HENRY FORD HOSPITAL MEDICAL BULLETIN

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SOME ASPECTS OF INSECT ALLERGY

JOSEPH H. SHAFFER, M.D.*

Within the past few years, numerous articles dealing with various phases of allergy to insects have appeared in scientific journals and lay press. In a dramatic fashion, they call our attention to the way in which insects cause allergic reactions which may at times pose a real threat to life.

An article appeared in the June, 1959, issue of *The Journal of Lifetime Living* entitled, "Is there a Killer in your Back Yard?"[1] and was also abstracted in the *Reader's Digest* that same month.[2] The author related in graphic detail his experience when stung by a swarm of angry wasps. This article listed six recent fatalities, in various sections of the United States, following insect stings in which sudden death occurred before medical aid could be summoned. These articles were called to our attention by telephone calls and letters. Inquires were mostly from persons who had experienced generalized symptoms following stings by insects; they were alarmed by these stories and were anxious to have treatment and, if possible, protection from serious reactions should they be stung again. Having been exposed to the body proteins contained in the insect venom at the time of a sting, such persons could well have become sensitized and are therefore candidates for anaphylactic response to subsequent stings.

Since insects have always been with us and are likely to continue as co-inhabitants on this earth, and since the health and life of certain sensitized individuals are constantly endangered by their presence, I thought it proper to review briefly the role that insects play in the production of allergic symptoms, along with some suggestions as to treatment. This problem is deemed of sufficient importance that the American Academy of Allergy has a committee to review and encourage research in this field.

EXPOSURE BY INHALATION

Allergic disorders of the respiratory tract have been associated with sensitivity to butterflies, caddis flies, moths, May flies, bees and other flying insects. Inhalation of scales, hairs, wing and body fragments, and other body emanations, given off in flight, are found in atmospheric dust when these insects are prevalent at certain seasons of the year. Persons may become sensitized to such substances and may experience conjunctivitis, rhinitis, bronchitis and bronchial asthma. Patients who suffer from pollen sensitivity may have identical symptoms. It is, therefore, necessary at times to consider insects in addition to pollens as etiological factors in the production of seasonal symptomatology.

Some thirty years ago, Figley[3] found certain patients living along the shores of Lake Erie near Toledo and Port Clinton, Ohio, who had symptoms from June to early August that were caused by May flies. Patients were subjected to conjunctival, inhalation and intradermal tests and were found sensitive to extracts of the pellicle given off during the moulting season. Pellicles were thin and friable, easily fragmented

*Division of Allergy.

by the wind, and could be carried long distances. Thus available for inhalation, they could and did produce allergic sensitization. Figley, in his studies, found extracts of dried whole caddis flies just as effective as pellicle extracts for testing and for desensitization. He reported complete relief from symptoms in his patients following desensitization. Others[4] have reported similar results in the treatment of caddis fly sensitivity.

EXPOSURE FROM BITES

Percutaneous instillation of salivary secretions during bites from chiggers, ants, mosquitoes, gnats, fleas, house-flies and other flies and biting insects produces local discomfort. Such bites may, however, cause marked erythema, swelling, pruritis, ecchymosis and angioneurotic edema, the latter of which may not appear for 24 to 48 hours.

One of my patients (M. LeF.), a white female attorney, age 63, with a negative history for other forms of allergy, experienced malaise, chills and fever, generalized urticaria, extreme weakness and collapse following the bite from a fly. The specimen brought to us was identified by an entomologist as "deer-fly", a Diptera of the Family of Tabanids."[5] Patients sensitive to this group of biting insects may be tested and treated with insect whole body extracts.

EXPOSURE FROM STINGS

The most important insects in the production of serious and potentially fatal anaphylactic reactions are the Arthropods of the Hymenoptera group. This group includes the honey-bee, bumble-bee, wasp, yellow and black hornet, and the yellow jacket. (Fig. 1.) A sting may cause intense localized redness, swelling and pain which may persist for several hours. Individuals may experience local reactions of diminishing intensity with subsequent stings due to natural immunization. Such repeated exposures to the body proteins present in the venom sac contents may, in other individuals, produce sensitization, or an altered reactivity on the part of body tissues. It is difficult to predict which symptoms may be produced by subsequent stings when such an allergic state exists.

SYMPTOMS

Symptoms may consist of one or more of the following: localized pain, swelling, generalized erythema, a feeling of intense heat throughout the body, headache, swollen and tender joints, generalized pruritis, urticaria, weakness, abdominal cramps, dyspnea, constriction of the chest, asthma, vascular collapse and possible death from anaphylaxis within a very few minutes. Such patients have on occasion been found dead — and erroneously recorded as death due to heart attack or sunstroke because the sting site had gone unnoticed.[6]

Barnard[7] reported three deaths from insect stings. One patient died in congestive failure and anaphylactic shock following stings from a wasp. Post-mortem examination on a second patient who died following stings from a yellow jacket showed visceral congestion and petechial hemorrhages with acute gastroenteritis and myocarditis. A third patient was stung by a bee and died of suffocation due to edema of the larynx.

STINGING INSECTS

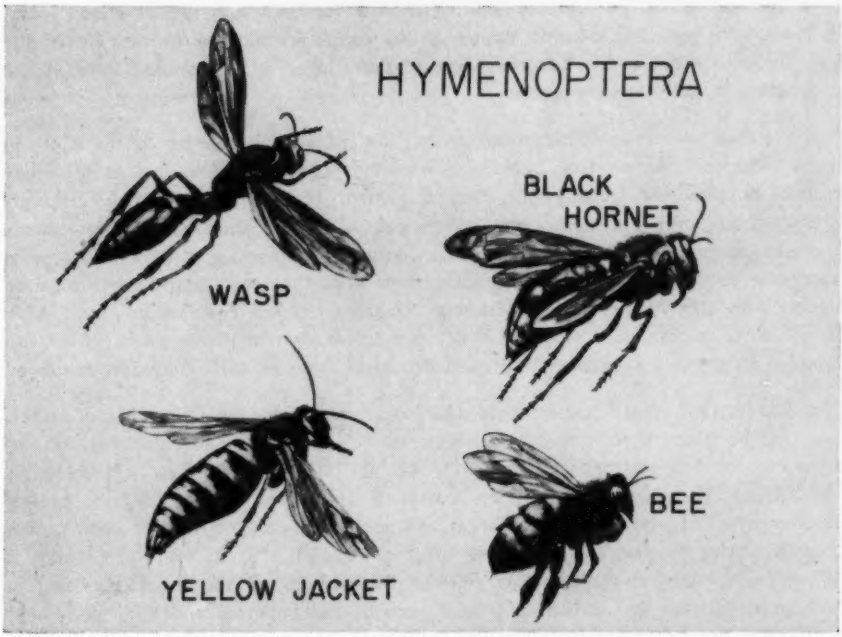

Figure 1

Serious and potentially fatal anaphylactic reactions may be caused by stings from the Arthropods of the Hymenoptera group of insects.
Drawings from *What's New*, No. 211, 1959, courtesy of Abbott Laboratories.

Insects of the Hymenoptera group, except the bee, retain their stinger and are in a position to sting repeatedly, thus causing multiple traumatic areas, each time injecting some portion of the venom sac contents. The honey-bee, however, leaves its barbed stinger at the site of the sting. Should the bee be brushed off, the venom sac separates from the body of the bee. Although separated from the body, the smooth muscles of the venom sac continue to contract for two to three minutes and each contraction forces some of the contents of the venom sac through the stinger shaft until the sac is emptied.

NATURE OF THE VENOM

The complex chemical substances found in the venom cause marked local irritative reaction, while the protein fractions of the venom may produce allergic response. Benson and Semenov[8] subjected bee venom to chemical analysis and were able to detect the presence of an indol derivative (probably tryptophan), choline, glycerol, phosphoric acid, palmitic and other fatty acids, and a non-nitrogenous substance thought to be saponin. Formic acid and histamine were not found. Saponin, or saponin-like substance, was thought to release histamine from the skin through

the interaction of dermolysin. They concluded that sensitization following a sting by a bee was caused by proteins inherent in both the body and the secretions found in the venom sac. Microscopic studies of the stinging technique and stinger of the bee failed to show pollen contamination of the stinger as a possible factor in the production of the allergic reaction.

Perlman[9] states that little is known of the nature and number of excitants in insect allergens that produce immediate whealing response and antibodies that may readily be passively transferred. The integument of the Arthropods is generally presumed to contain the allergenic substances. Chemical analysis of insect cuticle contains protein, carbohydrate and sulfur-containing compounds. Perlman prepares allergenic extracts from live or quick-frozen whole insects, defats the broken-up insects with ethylether, then extracts with isotonic buffered aqueous solution. After lyophilizing, the material is stored in dry powdered form in a dessicator. From this powdered antigen a glycerinated buffered extract is prepared for testing and treatment.

Foubert and Stier[10] prepared separate extracts from the pulped bodies of honey-bees, wasps, black hornets, yellow hornets and yellow jackets and compared antigens found in the five extracts. By employing the gel diffusion technique of Ouchertony they found that each of the insects contained from four to six antigenic protein fractions, two of which seemed identical and present in each of the five insects. The remaining two to four fractions were specific for each species, but were shared in common by wasps and honey-bees, but not by hornets and yellow jackets. Since a person may have been sensitized to only one of the antigens, and with subsequent stings may become sensitive to other antigens present, it is desirable to use an antigen mixture that will protect against all insects in this group. Although there is some difference of opinion among allergists as to whether venom sac contents alone, or extracts from the whole body of the insect should be used for diagnostic tests and treatment, it is generally accepted that insect whole body extracts are more potent antigenically and are the treatment material of choice.

TESTING AND DESENSITIZATION

It has been suspected that a refractory period of ten to fourteen days persists (as in experimental anaphylaxis) following an insect sting, during which time skin tests may be negative. Tests should therefore be delayed for several weeks after the stinging of the patient, and should be applied with extreme caution. It has been our experience, as it has with others, that patients sensitized to insect allergens may give a positive reaction to an intradermal test dose as small as 0.025 cc. of a 1:100,000,000 dilution of the extract. Should the initial test with this weak dilution fail to give a positive reaction, successively stronger test dilutions should be applied until a positive reaction is obtained. The test dose that produces a positive reaction could be considered as a relatively safe dose for starting desensitization. We use commercially prepared single insect extracts for testing, and a multiple whole insect extract mixture (containing honey-bee, wasp, hornet and yellow jacket) for desensitization.

*Hollister-Stier Laboratories.

Table 1
DESENSITIZATION WITH POLYVALENT INSECT ANTIGEN

Dosage Schedule*

Dilutions					
1-1,000,000	1-100,000	1-10,000	1-1,000	1-100	1-10
0.05 cc	0.05 cc	0.05 cc	0.05 cc	0.05 cc	0.05 cc
0.10	0.10	0.10	0.10	0.075	0.075
0.20	0.20	0.20	0.20	0.10	0.10
0.40	0.40	0.40	0.30	0.15	0.15
			0.40	0.20	0.20
				0.30	0.30
				0.40	0.40

*Given at intervals of 4 to 7 days until maximum tolerance dose is reached — then maintain at 3 to 4 week intervals.

Desensitizing treatments are given at intervals of seven days and in accordance with a graduated dosage schedule as recommended by the manufacturer.* (Table 1.) Dosages are regulated so as to avoid marked local reactions at the site of the injection which is given subcutaneously. We try to avoid causing a systemic reaction from over-dosage or by accidental injection into the blood stream. Should a reaction occur immediately or during the twenty minute observation period after treatment, a tourniquet is applied above the injection site and epinephrine hydrochloride 1-1000 dilution is administered in divided doses (0.20 to 0.30 cc.) and repeated as necessary until the reaction has subsided. We have been able to reach a dose of 0.40 cc. of 1-100 dilution. The patient is maintained on that level every two weeks for two to three months, then every three weeks for a few months, then continued on maintenance therapy every four weeks on a year round basis.

Mueller[6] reviewed 120 cases of allergy to stinging insects and found only three instances where capture and proper identification of the insect that delivered the sting had been possible. Educational programs along this line met with little success. He points out that knowledge of the nesting habits of these insects could be helpful. Small honey-comb type or mud nests of the wasp are found in protected areas around buildings, yellow jackets' nest under logs or rocks and in the ground, and the papier-maché foot-ball sized hornets' nest in bushes and trees. Bees are usually encountered around fields of clover. Unless the stinging insect can be positively identified, Mueller recommends broad protection by treating with multiple insect whole body extracts.

PROTECTIVE EFFECT OF DESENSITIZATION

Mueller[6] desensitized 76 patients who had experienced systemic reactions to stings. Thirty were re-stung and only one suffered systemic symptoms while on maintenance

therapy, or after having completed three years of desensitization treatment. (Table 2.)

Table 2
PROTECTIVE EFFECT OF DESENSITIZATION

	Before Treatment		After Treatment	
	Response to Stings			
Patients Reported	Systemic		Systemic	Local**
Mueller	76		1*	29
Jones and Laws	20		0	6
Swinny	41		0	19
Thomas	18		0	12

*Re-stung after treatment and experienced anxiety end itching.
**Local reactions—minimal to moderate.

Jones and Laws[11] reported their experiences with 20 patients who had experienced generalized allergic reactions to the venom of stinging insects. Six of these patients were re-stung while undergoing or after completion of desensitization therapy; they experienced moderate local reactions only at the sting site.

Swinny[12] states that desensitization can be effective and is often life-saving. He reported 41 patients who had anaphylactic types of reactions to insect stings; and 19 of this group were subsequently stung but had local reactions only.

Thomas[13] reports 12 of 18 patients who were hyposensitized and subsequently stung or bitten and none of whom suffered systemic or constitutional reactions, but had local reactions only. He recommends that all patients who have had severe local reactions as well as systemic reactions be considered for hyposensitization as they are potential candidates for severe systemic reactions, some of which may be fatal.

One of the four patients (Case 1) reported in this paper was re-stung while receiving maintenance doses of 0.20 cc. of a 1-100 dilution of polyvalent insect antigen every four weeks. He experienced a minimal local reaction only at the site of the sting.

EMERGENCY TREATMENT

If we are to effectively combat serious reactions caused by insects, it is imperative that we have a well thought out treatment plan to put into effect with the least possible delay. Our patients are furnished with an emergency kit and are instructed to keep it readily available. (Fig. 2.) It contains tourniquets (sufficiently long to encompass the thigh) to apply proximal to stings on the extremities, and tweezers for removing bee stingers, isoproterenol, 10 mg. tablets, for sublingual absorption, and epinephrine hydrochloride (Medihaler Epi) for rapid aerosol inhalation. The patient and members of the family are instructed in the administration of these preparations. Antihistamine ointments applied locally along with oral preparations have controlled itching and urticaria. Cold packs to the sting area have proved soothing and helpful in reducing local discomfort.

EMERGENCY TREATMENT FOR INSECT ALLERGY
1. Remove bee stinger with tweezers at once.
2. Dissolve white tablet (Isuprel) under the tongue.
3. Use inhaler (epinephrine) for difficult breathing.
4. Apply tourniquet on arm or leg above sting site.
5. Cleanse sting area with antiseptic towelette.
6. Apply cold pack to sting area.
7. Read instruction sheet carefully.
8. Call your doctor at once!
ANTISEPTIC TOWELETTE
EPINEPHRINE
AEROSOL

Figure 2

Emergency kit for insect sting.

First aid measures, as just mentioned, may prove sufficiently effective to counteract the effect of the insect venom. There are occasions, however, when the services of a physician are necessary to "take over" in the treatment of the violent generalized anaphylactic reactions which may prove fatal. Such a situation may require epinephrine hydrochloride in divided doses, intravenous fluids containing levarterenol bitartrate (Levophed), cardiac stimulants, and possibly epinephrine administration intracardially.

Ethylnorepinephrine hydrochloride (Bronkephrine) given hypodermically may be substituted for epinephrine, thus avoiding the pressor effect which may be undesirable in treating patients with hypertension or cardiac disease. Should swelling of the neck and throat, or edema of the larynx occur, it may be necessary to perform tracheotomy or to insert an airway for administration of oxygen.

CASE REPORTS

(Case 1.) R.P., a white male age 49, while working as a tree-trimmer for a local power company, was stung several times in quick succession by yellow jackets. Shortly thereafter he developed a severe and generalized rash. He was taken to the company doctor who administered adrenalin injections and antihistaminics. Twenty-four hours later the rash had cleared, and the doctor warned him to rush to the hospital for immediate treatment should he be stung again.

The patient's wife, in a letter to us, referred to the article in the *Reader's Digest* which stated that immunization injections could be given. Since her husband was repeatedly exposed to stinging insects in his work, they were greatly worried and were anxious that he receive immunization therapy. The patient was tested on September 9, 1959, and showed a 2 plus reaction to 0.025 cc. intradermal test dose of a 1-1,000,000 dilution of yellow jacket extract. He was started on treatment with dosages of this dilution, using a multiple whole insect extract. While on a maintenance dose of 0.20 cc. of a 1-100 dilution, given at intervals of four weeks, he stepped on a bee. He experienced minimal localized swelling only at the sting site.

(Case 2.) K.D., a white male student, age 18, was brought to the clinic by his father October 21, 1960. This boy had been stung several times by insects over the previous two years but had experienced no great difficulty until the summer of 1959. While on an automobile trip he was stung by a bumble-bee. Local swelling had persisted for several hours, but alarming symptoms developed within a twenty-four hour period which caused them to stop in a nearby village for medical assistance. The patient had high fever, chills, extreme weakness and became semi-delirious. Although the original sting site showed nothing, the left eye was completely closed by swelling. Symptoms cleared within 48 hours with treatment by the physician.

The patient was tested October 21, 1960, and was found sensitive to wasp, 1 plus, and negative to bee, hornet and yellow jacket by intradermal tests with 1-10,000 dilutions. A 2 plus positive reaction was obtained to 0.05 cc. intradermal test with a 1-100,000 dilution of mixed whole insect antigen. He was placed on treatment with this dilution as a starting dose and is now receiving his injections under the direction of his local physician. The patient was furnished a kit containing tourniquets, ephedrine sulfate Gr. 3/8 capsules, Isuprel (10 mg.) sublingual tablets. He was instructed in the use of the tourniquet, medications, and application of ice packs.

(Case 3.) A.B., age 46, a staff surgeon of our hospital, was seen in our clinic August 9, 1960. While working in his yard on August 7th, he was stung on the right knee by a "sweat bee." Although there was very little localized swelling, ten minutes later he developed generalized giant urticaria, the lips became swollen, and

there was difficulty in swallowing. He realized he was having a reaction and sought assistance from an internist who lived nearby. After treatment with divided doses of epinephrine hydrochloride, Depo-Medrol and oral cortisone, symptoms cleared.

The patient stated that bee stings over the years had caused no difficulty, but that bites from horseflies caused large welts on the skin. Six months before, he had a dermatitis on the wrists from printer's ink. The personal and family histories were otherwise negative for allergy.

On August 9th (48 hours after the allergic episode), scratch tests to wasp, hornet, bee and yellow jacket were negative to 1-100,000 dilution. Retesting was done on August 30, 1960, using mixed whole insect antigens of the above insects with the following results:

1-1,000,000 dilution — negative; 1-100,000 dilution — weak, one plus. 1-10,000 dilution, 4 plus with marked pseudopodial reaction. This patient was started on desensitization with a dose of 0.025 cc. of a 1-100,000 dilution of mixed whole insect antigen containing wasp, hornet, bee and yellow jacket.

(Case 4.) C.B., a white male, age 57, came to our clinic December 15, 1960, at which time the following history was elicited. While cutting grass at his cabin in Northern Michigan in September, 1960, he was stung on the abdomen by an insect which "appeared to have come out of a nest in the ground." There was immediate redness, swelling and pain at the sting site but in five minutes he was aware of tachycardia and vertigo which caused him to lie down. He became confused and semi-comatose but roused in an estimated twenty minutes and again started for his cabin. His wife was alarmed at his pallor and profuse sweating. He crawled into bed and covered himself and as he became warm he broke out with a generalized urticaria with associated swelling of the lips and tongue and there was difficulty in swallowing.

The patient was rushed to a clinic in a nearby town where he was given injections of epinephrine and Benadryl with effective relief. The physician furnished the patient with Isuprel linguets, syringe, needle and injectable Benadryl with instructions as to their use, should he be stung again.

Although the patient had been stung repeatedly in childhood this was the first time he had experienced generalized symptoms. There was a negative history for other forms of allergy. His mother however developed hives after eating certain foods.

Intradermal tests with insect whole body extracts were done on December 15 and 16 with the following results: mixed antigen (wasp, bee, hornet, yellow jacket) 1-1,000,000 dilution — negative; 1-100,000 dilution — negative; hornet — 1-100,000 dilution — negative; yellow jacket 1-100,000 dilution — 2 plus; honey-bee 1-10,000 dilution — 1 plus; wasp 1-10,000 dilution — 1 plus; hornet 1-10,000 dilution — 1 plus.

This patient was instructed in the use of an emergency kit with which he was furnished and was started on desensitization with mixed insect whole body extracts in a 1-100,000 dilution.

SUMMARY

1. Symptoms caused by various kinds of insects are reviewed. Inhalation of body proteins and emanations of butterflies, caddis flies, moths, May flies, and bees and other flying insects may cause allergic symptoms of the respiratory tract. Protection may, in most instances, be furnished by desensitization with whole insect body extracts.

2. Bites from insects such as chiggers, ants, mosquitoes, gnats, fleas, houseflies and other flies may cause local trauma only, or local and systemic allergic symptoms. Desensitization with whole insect body extracts may again be helpful in prevention of such allergic symptoms.

3. The most violent allergic reactions are caused by stings from insects of the Hymenoptera group (honey-bees, bumble bees, wasps, yellow and black hornets and yellow jackets). Persons who have been stung and have experienced mild to moderate local reactions only, may not need desensitization. Those persons who have experienced severe local and/or generalized allergic reactions from previous stings should, however, receive desensitization therapy with polyvalent, or mixed whole insect body extracts.

4. Although desensitization is effective in approximately 95% of the patients treated, it is well to furnish all patients who are allergic to insects with an emergency kit along with specific instructions as to its use. This kit should be readily available to the patient whether at home, on vacation trips, or in pursuit of daily activities.

5. Sudden death from anaphylaxis is a real threat to human life when persons, once sensitized to the protein substances of stinging insects, are re-stung. Effective emergency treatment and desensitization therapy can, in most instances, nullify this threat to life.

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THE FEEDBACK CONTROL OF CHOLESTEROL BIOSYNTHESIS*

W. T. BEHER**, G. D. BAKER**, W. L. ANTHONY**, AND M. E. BEHER**

The 29 carbon alicyclic alcohol, cholesterol, participates in the metabolic reactions of a wide spectrum of cells. It is a precursor of the steroid hormones synthesized by ovarian, testicular, placental, and adrenal cortical tissues[1]. It is rapidly metabolized by liver cells to bile acids, which in turn have important influences on intestinal absorption[2,3]. Cholesterol has been implicated in the transport of unsaturated fatty acids in the blood and in the regulation of the transfer of fatty acids across the cell membrane[4]; its role in the structure and metabolism of the nervous system has received at least preliminary investigation[5]. Although these functions are obviously vital to the metabolic and structural integrity of the living organism, it is the abnormally high blood and tissue cholesterol levels found in the disease, atherosclerosis, that is responsible for the present vigorous research interest in cholesterol metabolism.

Since we are concerned with the etiology and reduction of abnormally elevated tissue cholesterol levels in atherosclerosis, it would seem that basic research on the mechanisms by which various tissues maintain normal cholesterol levels should be of prime importance. Such studies necessarily involve factors influencing and controlling the relative rates of cholesterol anabolism, catabolism and distribution in many tissues. As an example, consider a very simple case: the ability of a given tissue to maintain a static cholesterol level in an isolated system such as a tissue slice or a homogenate. In such a system, the rate of synthesis simply must balance the rate of degradation; i. e. if the rate of degradation is increased, the rate of synthesis must increase equally or a net decrease in cholesterol level will take place. In the intact animal such a simple system does not exist. Here we must consider not only relative rates of anabolism and catabolism but also such factors as level of dietary cholesterol, rate of exchange of tissue and organ cholesterol with the blood, rate of enterohepatic recirculation, intestinal absorption, availability of cholesterol precursors, availability of certain coenzymes, and possibly the existence of active and inactive forms of cholesterol[6]. Since various hormones can cause alterations in the relative contributions of these and other parameters, we can see the enormous complexity of the problem. Obviously in any systematic study of these phenomena, one aspect at a time should be investigated while attempting to maintain a steady-state with respect to other variables. The following discussion will be limited to a consideration of the control of cholesterol biosynthesis by cholesterol itself and by the bile acids.

CHOLESTEROL FEEDBACK INHIBITION

Since enzymatic reactions may be retarded by (a) specific reaction product inhibitions or (b) reversal by accumulated reaction products, one would expect that cholesterol formation would be controlled by cholesterol or intermediates in cholesterol synthesis. A number of papers have appeared which show that, in various species,

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**Edsel B. Ford Institute for Medical Research, Biochemistry Department.

increases in liver cholesterol levels brought about by dietary cholesterol supplements, lead to striking decreases in the ability of the liver to synthesize cholesterol[7-17]. Conversely, decreasing hepatic cholesterol levels by bile duct cannulation[18], triton[19,20], lymph duct cannulation[21,22], x-radiation[20,23], or hyodeoxycholic acid[24] results in many fold increases in hepatic cholesterol synthesis rates, despite the strikingly different mechanisms involved. In studies on the effects of some of these procedures[12,24,25] in various tissues, it appeared that, with the exception of the adrenal, they had little, if any, effect on extrahepatic cholesterol synthesis rates. This suggests that these rates are controlled by factors other than those controlling liver cholesterol synthesis. In mice or rats fed cholesterol or hyodeoxycholic acid[26], we detected little change in cholesterol levels in tissues other than liver and blood. Recently, however, Morris and Chaikoff[27], employing a different approach to this aspect of the problem, concluded that dietary cholesterol does have an effect on extrahepatic cholesterol synthesis in all tissues studied except testes. Further experiments will be necessary to clarify this problem, as the details of cholesterol synthesis in extrahepatic tissues may differ significantly from those encountered in liver, and few studies have been made of the sequence of reactions in these tissues.

Frantz *et al*[28] showed that there is an inverse relationship between the logarithm of the rate of cholesterol biosynthesis and the total cholesterol concentration in rat liver slices. Gould[29] refined this work and found that the rate of hepatic cholesterol synthesis is more directly related to free cholesterol concentration than to total cholesterol. Neither of these approaches however explains the rapid changes in cholesterol synthesis rates obtained during the initial phases of cholesterol administration[30] or lymph duct cannulation[21,22], which are unattended by measurable alterations in hepatic cholesterol concentration. It might be suggested that the changes in cholesterol synthesis are probably more related to the cholesterol concentration in or at the surface of specific cellular particulates than to the concentration of ester, free, or total cholesterol in a given liver.

Recently Siperstein[31] has shown that the addition of emulsions of cholesterol or cholesterol esters to rat tissue slices, synthesizing cholesterol from acetate-1-C^{14}, has little if any effect on the synthesis rate as long as the cholesterol concentrations are held within physiological limits. The validity of these *in vitro* experiments may be questioned on the grounds that there is some doubt whether the cholesterol or cholesterol ester in the artificial emulsions used in this study were able to penetrate or permeate cell or particulate membranes. Studies using either lipoproteins rich in cholesterol or a series of different types of emulsions would increase the value of these observations.

SITE OF CHOLESTEROL FEEDBACK INHIBITION

Since the sequence of reactions involved in the synthesis of cholesterol from acetate (with the exception of those reactions between lanosterol and cholesterol) are rather well defined (Fig. I)[32,33], and since several C^{14}-labeled compounds in the sequence are available, attempts have been made to pinpoint the reaction inhibited by cholesterol. In an *in vitro* investigation, Gould and Popjak[34] found that homogenates prepared from livers of cholesterol-fed rats, incorporated acetate-1-C^{14} into

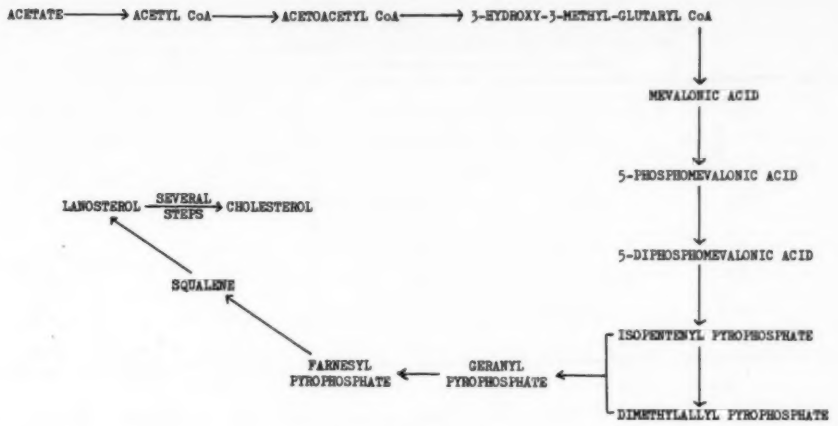


Figure 1
Pathway of cholesterol biosynthesis from acetate.

cholesterol much more slowly than homogenates from normal rats, while the rate of incorporation of mevalonic acid-2-C^{14} was not affected. On the other hand inhibition between mevalonic acid and cholesterol has been detected in other investigations. Siperstein and co-workers[30,35,36] found an inhibition of 30 to 40% in liver slice experiments; while Bucher *et al*[20] detected somewhat more inhibition in homogenates. The latter observations, however, represent only two tests. Bucher and co-workers also investigated the effect of cholesterol on the conversion of squalene to cholesterol, and detected some inhibition. This observation is not in agreement with Siperstein's experiments[31]. Study of cholesterol synthesis rates in microsomes and supernatant fractions isolated from homogenates derived from livers of normal and cholesterol-fed rats led to a series of interesting observations[20]: (a) cholesterol synthesis from acetate or mevalonic acid depends on the presence of both microsomes and the supernatant fraction; (b) combination of the supernatant and microsomes from liver homogenates derived from cholesterol-treated rats results in very little cholesterol synthesis from acetate; (c) combination of liver microsomes from cholesterol-treated rats with liver supernatant from normals results in very little cholesterol synthesis; (d) combination of liver microsomes from normal rats with liver supernatants derived from cholesterol-treated rats results in a near normal rate of incorporation of acetate into cholesterol. Thus, the effect of cholesterol on cholesterol synthesis influences some parameter carried in the microsomes. This may be a series of enzyme reactions, a single reaction, or a biophysical change in microsomal structures and/or permeabilities.

In an effort to study some of these effects in *in vivo* systems, our group investigated the influence of cholesterol feeding on the incorporation of acetate-1-C^{14} and mevalonic acid-2-C^{14} into mouse liver cholesterol. The results, shown in Table I, indicate that while cholesterol synthesis from acetate-1-C^{14} is depressed 95%, mevalonic acid-2-C^{14} incorporation is depressed 50% in the several groups. Purification of the digitonide precipitate fraction via the dibromide[37], which removes digitonin

Table I

Influence of Diets Supplemented with 1% Cholesterol on Mouse Liver Cholesterol Levels and Synthesis Rates as a Time Function

Diet Interval	Liver Cholesterol Level	Acetate-1-C^{14} Incorporation	Mevalonic Acid-2-C^{14} Incorporation	
			Before Dibromide Purification	After Dibromide Purification
Days	mg/g	Cholesterol counts per minute per gram tissue		
0	4.22 ± 0.9	2,496	22,729	19,774
4	8.81 ± 1.87	125	11,106	5,886
14	12.3 ± 2.85	135	8,703	4,265
21	15.2 ± 4.14	129	10,496	5,248

precipitable cholesterol precursors, results in a further loss of activity, bringing the inhibition of mevalonic acid-2-C^{14} incorporation to about 75%. This still may not be a maximal figure, for the dibromide purification is not quantitative[37]. Moreover, the control group of animals, with which the cholesterol-fed group was compared, may have totally exhausted the small amount of injected mevalonic acid-2-C^{14} by conversion to cholesterol during the one-hour incorporation. While further investigations will be necessary to establish its full extent, obviously the inhibition of mevalonic acid-2-C^{14} incorporation, while not as large as that of acetate-1-C^{14}, is substantial. The inhibition must lie in the steps from lanosterol to cholesterol, since dibromide purification, which removes digitonin precipitable 3β-hydroxysterols from lanosterol through desmosterol, results in a loss of C^{14} activity. Therefore the metabolism of some cholesterol precursor between squalene and cholesterol is being inhibited. Gould[29] demonstrated inhibition of the conversion of mevalonic acid to cholesterol by cholesterol *in vivo* in rats, but did not purify the isolated cholesterol digitonide.

The effects of some of the possible intermediates between mevalonic acid and cholesterol on biosynthesis of cholesterol from acetate have also been investigated. Feeding squalene, lathosterol, or 7-dehydrocholesterol inhibits cholesterol synthesis[11,13], while lanosterol and farnesol are ineffective. Farnesoic and geranoic acids, which are probably products of an alternate pathway of squalene metabolism, inhibit the conversion of mevalonic acid and farnesyl pyrophosphate to sterols[38]. Farnesoic acid inhibits the conversion of mevalonic acid to 5-phosphomevalonic acid[39]. Therefore the formation of these acids has been suggested as a possible control mechanism in sterol synthesis[32].

The foregoing observations have been interpreted in two ways: (a) Since the inhibition of cholesterol synthesis by feedback is much greater from acetate than from mevalonic acid or squalene, the main inhibition must lie prior to mevalonic acid; and further since acetyl Co A, acetoacetyl Co A, and hydroxymethylglutaryl Co A are all involved in vital reactions other than cholesterol syntheis (Fig. 2), which proceed uninhibited during the feedback, the only reaction where inhibition is likely is the conversion of hydroxymethylglutaryl Co A to mevalonic acid, a relatively nonreversible reaction. Lynen and coworkers[40] showed that this reaction was inhibited under feedback

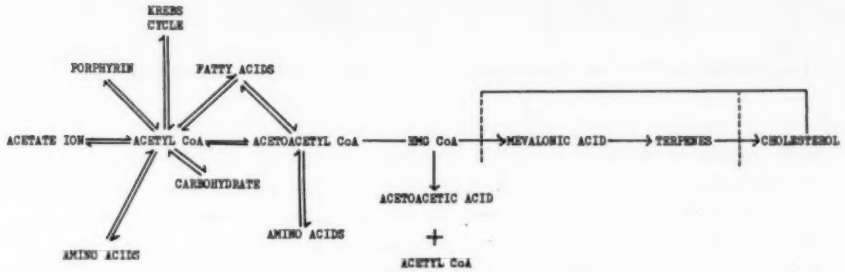


Figure 2

Metabolic interrelationships in cholesterol feedback inhibition.

conditions. (b) It is possible to regard the smaller feedback inhibition, mevalonic acid ——/——/→ cholesterol, as very important. The reaction sequence acetate ——→ mevalonic acid ——→ cholesterol, can be divided as follows: The metabolites prior to mevalonic acid are readily interconvertible (Fig. 2), and in two cases, viz. acetyl Co A and acetoacetyl Co A, are among the substrates most in demand for a variety of major synthesis pathways. In contrast, mevalonic acid seems to have little future save incorporation into isoprenoid compounds and subsequently into the steroid nucleus. That which can not be utilized is excreted unchanged.[41] Therefore during feedback inhibition, a uniform feedback all along the sequence of reactions would *appear* much greater if studied with acetate-1-C^{14} than it would *appear* if studied with mevalonic acid-2-C^{14} because the acetate-1-C^{14} would disappear more rapidly into other major pathways while the mevalonic acid-2-C^{14} would be rapidly converted to cholesterol*.

BILE ACID FEEDBACK INHIBITION

The feedback reaction we have been considering up to this point may be indicated in the following way:

i.e., cholesterol prevents its own synthesis by inhibiting a reaction or reactions between acetate and cholesterol.

Cholesterol is eliminated from an organism in a number of ways: (a) excretion in the fecal sterol fraction, (b) conversion to bile acids followed by fecal elimination, (c) loss by exfoliation of tissues containing the sterol, (d) metabolism to the steroid hormones with subsequent excretion, and (e) excretion in the sebum. Pathways (a) and (b) account for most of the elimination. If we once again turn to the concepts of product inhibition and reaction reversal, it might be expected that the fecal sterols and bile acids would limit the conversion of cholesterol to these endproducts by feedback reactions, such as the following:

*Only one isomer of dl-mevalonic acid is utilized for steroid synthesis. Therefore a maximum of 50% dl-mevalonic acid could be converted to cholesterol.

With respect to feedback *b*, the fecal sterols have an extrahepatic origin. They may be synthesized in several extrahepatic tissues, but the small intestine is the probable point of origin of the bulk of these substances. Recently Danielsson[42] has suggested that the synthesis of these sterols may be controlled by a feedback reaction similar to *b*. Reaction *c* has been investigated at least preliminarily. Eriksson[43] found that the normal rat produces around 5 to 6 mg per day of bile acid. If a tube is placed in the bileduct of a normal rat and led to the outside so that the bile acids which normally enter the enterohepatic recirculation are eliminated (bile duct fistula), the rate of bile acid production increases 10 fold. This is indirect evidence inasmuch as an animal under these conditions is abnormal. Bergström and Danielsson[44] more recently have shown that if one administers taurochenodeoxycholic acid (one of the two main bile acid conjugates of rat bile) to bile fistula rats, the bile acid excretion is lowered from around 60 mg per day to between 5 and 10 mg per day.

We have studied this feedback reaction in intact mice with elevated hepatic cholesterol levels[45]. Cholesterol-4-C^{14} was administered orally until the specific activity of the liver cholesterol pool was constant and liver cholesterol levels elevated. Feces were collected and the C^{14} activity of the bile acid and sterol fractions determined as a function of time. Cholic acid (Fig. 3) retarded the excretion of bile acids at all time intervals. There was little or no effect on the fecal sterol excretion. While the animals used in this study were intact, they did have elevated hepatic cholesterol levels and there is some doubt that this pool is representative of the pool in normal animals because of the presence of a high concentration of cholesterol esters[46]. We therefore investigated the effect of cholic acid on the fecal bile acid output in normal animals receiving a single injection of mevalonic acid-2-C^{14}. The results showed that cholic acid inhibits bile acid production by about 50% in the normal mouse[47]. Whitehouse and Staple[48] studied the inhibition of cholesterol degradation *in vitro*, and found that $C^{14}O_2$ production from cholesterol-26-C^{14} metabolized by a liver mitochondrial suspension, is retarded by the taurine and glycine conjugates of cholic acid. Danielsson[49] has recently pointed out that many reactions occurring during the conversion of cholesterol to bile acids in mitochondrial suspensions *in vitro* are due to auto-oxidation, are non-enzymatic, and do not correspond to *in vivo* reactions. Therefore observations on the production of $C^{14}O_2$ from cholesterol-26-C^{14} are suspect when applied to rate studies.

Scant attention has been given to the site of bile acid inhibition of reactions in sequence *c* from cholesterol to bile acids. Whitehouse and Staple[48] found that the metabolism of 3*a*, 7*a*, 12*a*-trihydroxycoprostane-27-C^{14} (an intermediate between cholesterol and the bile acids) measured by $C^{14}O_2$ production, was inhibited less by tauro- and glyco-cholic acids than the metabolism of cholesterol-26-C^{14}. However, as mentioned above, doubt can be cast on the validity of using $C^{14}O_2$ as an indicator of bile acid formation.

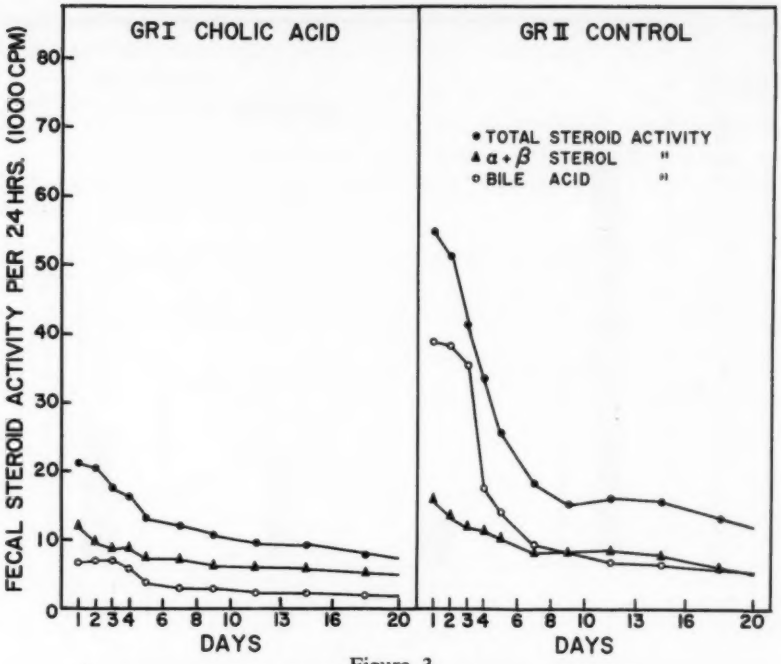
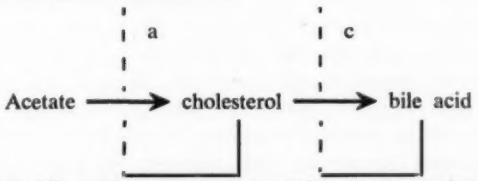


Figure 3
Fecal excretion of C^{14}-labeled steroids in normal and cholic acid treated mice with elevated tissue cholesterol-4-C^{14}

The use of free bile acids in metabolic studies has been questioned by Bergström[50]. He points out that since free bile acids are rapidly conjugated with taurine or glycine in the liver, their use may exhaust the supply of sulfur-containing amino acids, glycine, and serine, and thus upset normal hepatic metabolic patterns. While this is undoubtedly true, recent *in vivo* experiments[51] (Fig. 4) indicate very little difference between the effects of free and conjugated bile acids on cholesterol metabolism when administered at the 0.5% level.

THE DOUBLE FEEDBACK INHIBITION OF CHOLESTEROL BIOSYNTHESIS

Thus far we have been considering feedback reactions involving cholesterol or the bile acids as separate entities. However, if these two reactions are coupled a double feedback reaction (shown below), which may be important in controlling cholesterol metabolism, can be visualized.



Here an increase in bile acid concentration would retard reaction sequence *c* leading to an increase in the cholesterol level, which would in turn inhibit reaction sequence *a*.

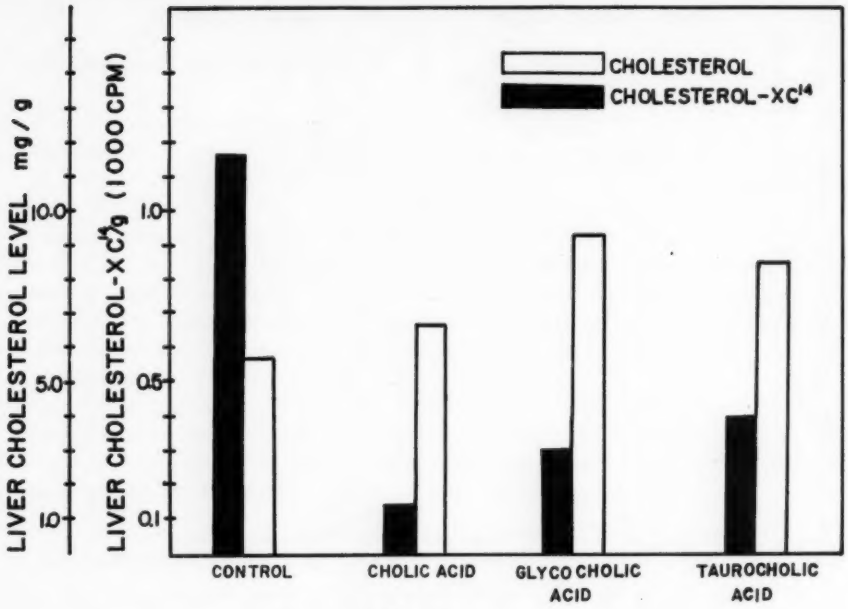


Figure 4
The effects of free and conjugated cholic acid on mouse liver cholesterol levels and synthesis rates (cholesterol-x-C^{14} levels).

Such a feedback system presumably would be effective only in the liver, since cholesterol oxidation to bile acids is only significant in hepatic cells[50] and feedback reaction *a* appears to occur only in the liver and possibly the adrenals.

The following experimental evidence supports the double feedback hypothesis. Swell *et al*[18] noted that 24 hours after cannulation of the bile duct of rats, cholesterol synthesis was increased 10 fold. Inasmuch as there is about a 10 fold increase in bile acid production at the same time[43], this is at least indirect evidence in favor of the double feedback reaction. Work done by the authors[34,45, 52-55] has provided considerable evidence in favor of this sequence. The previously discussed experiments, illustrated in Figure 3, show that cholic acid inhibits reaction sequence *c*, the conversion of cholesterol to the bile acids. If the double feedback hypothesis is correct, cholic acid administration should lead to increases in hepatic cholesterol levels and decreases in liver cholesterol synthesis rates. To investigate this point, mice or rats were fed basal diets supplemented with cholic acid, taurocholic acid or glycocholic acid. The animals received intraperitoneal injections of acetate-1-C^{14}, were sacrificed and liver cholesterol and cholesterol-x-C^{14} levels determined. The results (Fig. 4) show that cholic acid and its glycine and taurine conjugates increase liver cholesterol levels and decrease the rate of cholesterol synthesis (inhibition *a*). That the decrease in cholesterol synthesis was due to the increased level of hepatic cholesterol was shown by a further experiment (Fig. 5). When liver cholesterol levels, synthesis rates and bile acid levels were determined as a function of time, increases in bile

acid and cholesterol levels were in phase and correlated well with decreases in hepatic cholesterol synthesis rates (cholesterol-x-C^{14} levels).

If the double feedback reaction is valid, cholic acid or its conjugates should not inhibit directly at *a*. This point was investigated by Siperstein[31] who demonstrated that *in vitro* cholesterol synthesis in rat liver slices is not inhibited by cholic acid or its conjugates *per se*. Taken as a whole, there is considerable evidence in favor of the double feedback reaction.

APPLICATIONS OF THE FEEDBACK INHIBITIONS

Various attempts have been made to apply these basic findings to the problem of control of tissue cholesterol levels. It has been demonstrated, for example, that

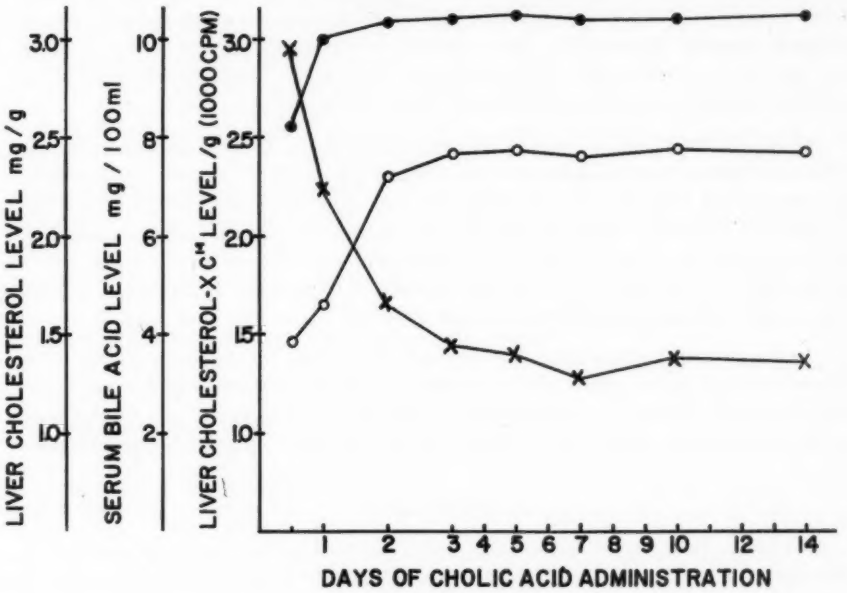


Figure 5
The effect of cholic acid on cholesterol, cholesterol-x-C^{14}, and serum bile acid levels as a function of time.
● Liver cholesterol. O Serum bile acid. χ Liver cholesterol-x-C^{14}

intestinal micro-organisms have important effects on bile acid turnover rates. Gustafsson and co-workers[56,57] found that in germ free rats the normal bile acid turnover rate of 5 to 6 days is doubled. Under germ free conditions, rat blood cholesterol is elevated[58]. Various antibiotics, which alter and decrease intestinal flora concentrations, increase serum cholesterol levels[50, 59-63], presumably by decreasing the conversion of cholesterol to bile acids.

Attempts have been made to lower blood cholesterol levels by increasing bile acid excretion rates, and in turn increasing cholesterol catabolism. Siperstein *et al*[64,65] observed that diets containing 3% ferric chloride (which precipitates bile acids *in vitro*)

limited the rise in blood cholesterol and the severity of atherosclerosis in cholesterol-fed cockerels. More recent experiments[66-68] show that ferric ions are ineffective in preventing cholesterol absorption or increasing the rate of conversion of cholesterol to bile acids in mice and rats. Setty and Ivy[68] studied the effect of aluminum phosphate gel in rats and found no significant increase in bile acid excretion. Rodbard[69] had earlier reported a decrease in blood cholesterol and atherosclerosis in chickens fed similar gels. In any case, the use of metal ions is limited by toxicity. Effects of anion exchange resins, which sequester bile acids *in vitro* systems, have been investigated *in vivo*. Tennent *et al*[70] showed that MK-325 and MK-135, (polymeric organic bases) inhibit blood cholesterol increases and aortic plaque formation in cholesterol-fed cockerels. These resins also lower blood cholesterol levels and increase fecal sterol and bile acid excretion in dogs. Feeding MK-135 to rats did not lower blood cholesterol, possibly because of the relatively greater ability of the rat to synthesize hepatic cholesterol[71]. Setty and Ivy[68] investigated several compounds of this type; most increased bile acid excretion to some extent. MK-135 has been found to induce steatorrhea in human subjects[72].

Hyodeoxycholic acid is another interesting substance. This compound is unique in that it effectively lowers blood and liver cholesterol levels[24,73-75] in mice by increasing the excretion of fecal sterols[45]. In this case part *c* of the double feedback reaction is bypassed. In normal mice, hyodeoxycholic acid or its glycine conjugate increases fecal sterol output about 3 fold[47]. Hyodeoxycholic acid has no demonstrable effect on rat hepatic cholesterol synthesis rates[47], while it increases this parameter in mice 3 to 6 fold[31]. A species difference appears to exist.

Little is known concerning the role of defects in the feedback reactions in various diseases which affect cholesterol levels. Recently Dubach and co-workers[76] investigated this problem in experimentally nephrotic rats, and found that the feedback mechanism responds to dietary cholesterol in an essentially normal fashion in these animals.

CONCLUSIONS

1. It has been shown by various approaches that in hepatic tissue the rate of cholesterol biosynthesis is controlled by the cholesterol level. There is conflicting evidence concerning the effect of cholesterol on cholesterol synthesis rates in extra-hepatic tissues.

2. The feedback inhibition of cholesterol synthesis may take place at several steps in the cholesterol synthesis sequence. It appears that the major point of inhibition is at the conversion of hydroxymethylglutaryl Co A to mevalonic acid; however, strong inhibitions are also effected between mevalonic acid and cholesterol.

3. The rate of conversion of cholesterol to bile acid is also controlled by feedback inhibitions, but few studies have been reported in connection with the site of inhibition in this sequence of reactions.

4. The cholesterol feedback reaction and bile acid feedback reaction are coupled in *in vivo* systems, resulting in a double feedback reaction sequence. Study of factors affecting this double feedback reaction may help to explain abnormal cholesterol

levels in various metabolic disturbances, and offers a promising approach to the problem of controlling tissue cholesterol levels.

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EXAMINATIONS OF HIPS IN NEWBORN BABIES AND RESULTS*

STANKO STANISAVLJEVIC, M.D.**

In the last three years the author examined personally 4300 newborn babies between the second and sixth day of age. From this group 2920 were females and 1380 males. Of the females 2514 were white and 406 were colored and of the males 1195 were white and 185 colored. The newborn was examined in the nursery and the follow-up done in the Pediatric and Orthopedic Clinics.

METHOD OF EXAMINATION

The newborn was not examined on the day of birth, in order to avoid additional stress to the baby.

The examination consisted of:

a) Observation of the lower part of the body and lower limbs.

b) Manual examination of the hips.

c) Radiographic examination.

A) Observation of the lower part of body and lower limbs.

It was noted that the newborn baby while supine and without diaper kept the lower extremities in the various positions. (Figure 1). Only the breech born baby held the thighs in abduction of 70-85°, and flexed at 95-100°. The knees were flexed at 90-100°. The skin of the lower part of both the gluteal and perineal regions were cyanotic color. (Figure 2). The breech born baby held the lower limbs in this position for 5-10 days and the color of the skin persisted for the same time.

The symmetry of the skin folds of the gluteal, perineal and thigh regions were observed. In our series we detected asymmetry of the skin folds of the gluteal region in 12 babies (8 females and 4 males); in the perineal region in 11 babies (9 females and 2 males); on the medial and posterior aspect of the thigh in 37 (29 females and 8 males). In 7 babies (6 females and 1 male) the skin folds were asymmetrical in all three regions. The manual and radiographic examinations of the hips in these newborn babies were negative. The babies were followed for a period of 3-14 months and in no case developed congenital hip dislocation (C.H.D.). Usually the asymmetry of the skin folds disappeared after 2-3 months, except in 4 cases of the last group in which the asymmetry of the skin folds persisted for 12-14 months, without any sign of C.H.D. (Figures 3, 4, 5, 6, 7).

B) Manual examination of the hips[7].

To examine properly the hips of the newborn the diaper should be removed and the baby laid on a firm examining table with enough space for manipulation of both hips.

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*This work is a part of the paper awarded the First Prize by the Detroit Academy of Orthopedic Surgery in 1960.

**Department of Orthopedic Surgery and Orthopedic Research Laboratory.

Figure 1

One of the most common spontaneous positions of the lower extremities in the newborn baby while supine.

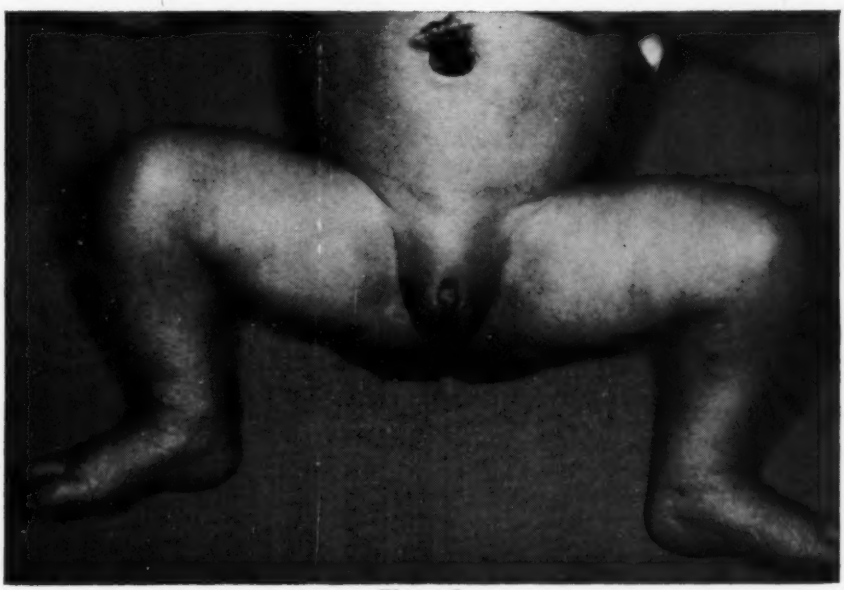

Figure 2

Characteristic spontaneous position of the lower extremities in the newborn baby delivered in breech position. Note the dark color of the lower gluteal and perineal regions.

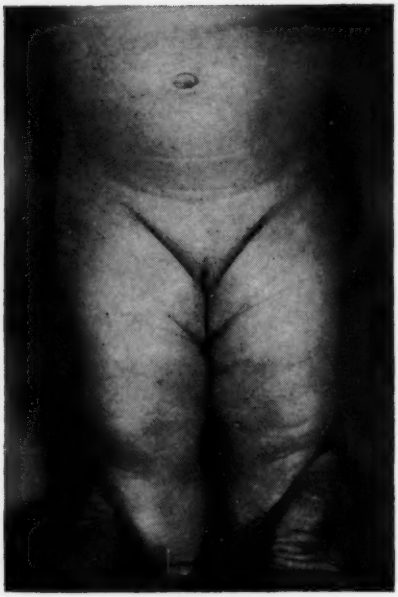

Figure 3

Baby girl who had at birth asymmetrical skin folds in the perineal, gluteal regions and on the medial aspect of the thighs. Photo taken at 10 months of age. At 14 months the asymmetry of the skin folds disappeared spontaneously. Manual and radiological examinations of the hips were negative since birth.

Figure 4

The same patient with asymmetrical folds on the gluteal region and on the thighs.

Figure 5

The same patient with full range of the abduction of the thighs.

Figure 6 and 7

Radiographies of the pelvis of the same patient. Anterio-posterior and frog-leg views disclosed normal hips.

Ortolani's[4,5] or the sign of "click" can be produced in the newborn baby with congenital hip predislocation (C.H.P.) only if the exact technique of examination is applied (Figure 8).

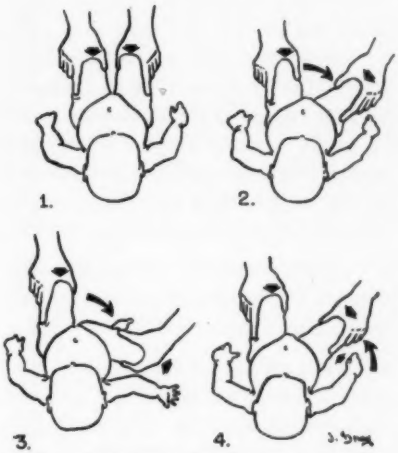

Figure 8

Four phases of the hip examination in the newborn baby searching for Ortolani's sign.

While the child is supine both hands of the examiner hold the baby's lower extremities around the knees. The thumbs are placed on the medial side and the other fingers on the lateral side. The index finger is placed along the femur shaft with the tip on the region of the great trocanter. The right hand of the examiner is on the left knee and vice versa. The thighs are flexed at 90° in neutral rotation and the knees are flexed at 90°-100°. To examine the left hip, the examiner holds the right hip firmly against the examining table in the above manner. The examiner exerts moderate pressure on the left knee downward to transmit pressure to the femur and hip.

In the case of C.H.P. on the left side, the examiner should feel a very slight "click" produced by the passing of the head of the femur over the ridge of the acetabulum posteriorly. Keeping the same amount of pressure the examiner begins to abduct the left hip and at approximately 45-50° of abduction a certain resistance should be felt, but this is not a true limitation of abduction. At that moment pressure of the tip of the index finger is exerted upon the region of the left great trocanter and the hip is reduced. At that moment another sensation of the "click" should be felt, and there is no further feeling of resistance to the abduction which was present prior to the hip reduction. The right hip is examined in the same manner. The newborn babies were examined also to establish the reverse hip-knee-hip triad which has been described by the author.[8]

C) Radiographic examination was performed for every newborn baby with positive Ortolani's sign and in an additional 350 newborn babies whose hips were completely normal clinically.

DISCUSSION

A) Every textbook of fundamental orthopedics describes asymmetry of the

gluteal, perineal or thigh skin folds as one of the signs which should make us suspect C.H.D. in children.

We limited our study to newborn babies only, and our experience shows that asymmetry of the skin folds was not reliable in the diagnosis of C.H.D. or C.H.P. We found asymmetry of the skin folds in the newborn baby who had much subcutaneous fat. This asymmetry disappeared after a few months except in four cases and may be due to the formation of small subcutaneous fibrous bands which persisted for longer period. These four babies had an unusal amont of subcucutaneous fat on the lower part of the body and the lower extremities from birth until 12-14 months of age when they began to walk. At this time we made the final clinical and radiographical examination which established the finding of normal hips.

Special attention was paid to those newborn babies who delivered in the breech position, a controversial cause of C.H.D. and C.H.P.[3]. In our series there were 215 breech born babies, and in only one a positive Ortolani's sign was detected.

B) The exact technique must be applied in the examination of the hips in the newborn babies in whom we are looking for the positive Ortolani's sign. If the positive Ortolani's sign is present, the "click" should be felt with the hands more than heard. The first "click" is simultaneous with a feeling of the femoral head passing over the ridge of the acetabulum and a feeling of reduction with the second "click". We are convinced that the "click" could be very easily misinterpreted if all details of the examination are not known.[1] The audible "click" which does not represent a positive Ortolani's sign in the newborn babies can be produced by the following anatomical structures.

1) The interposition of the ligamentum teres between the head of the femur and acetabulum in the supero-posterior part of the acetabulum.

2) The "click" in the knee during the examination of the hips could be interpreted as the "click" of the hip.

3) Gliding of the tendon of the ileopsoas could give the "click" during the examination of the normal hips in a case of an unusually large ileo-pectineal burso.

4) Gliding of the tendon of gluteus maximus and tensor fascia lata over the great trocanter can produce the "click" in the examination of the hips in the newborn babies.

These observations are the result of clinical examinations and anatomo-pathological dissections of 115 still born babies.[9]

In our series we detected the positive Orotolani's sign in 22 newborn white babies. Of these 9 had bilateral and 13 unilateral positive Ortolani's sign; 10 on the left and 3 on the right. Among them 19 were females and 3 males. There was no sign of C.H.P. in the newborn colored babies.

Ortolani's sign showed interesting changes with the growth of the four baby girls in whom it was detected at birth on the left hip and for whom treatment was started two months after birth. In those babies the "click" of Ortolani's sign was

more audible at three weeks of age than immediately after birth. The feeling of resistance prior to reduction, which in the newborn is vague, is more developed in the three week old baby. Those babies at two months of age and still untreated showed the first "click" still audible, but the second was not easily produced because of limitation of abduction which started to develop.

The author would like to emphasize that in the newborn babies with the positive Ortolani's sign limitation of abduction of the hips does not exist. The examiner looking only for limitation of abduction of the hips with no thought of Ortolani's sign will completely miss the hips in the phase of predislocation.

The positive Ortolani's sign found at birth disclosed that the hips of the newborn were in the phase of predislocation at birth. It is impossible to predict whether this condition will evolve into C.H.D. or C.H. subluxation. Spontaneous healing of hip dislocation has been widely discussed.[2] In our series we did not find spontaneous healing of C.H.P.

C) Radiography of the hips of the newborn babies with positive Ortolani's sign revealed the acetabular angle greater than 28°. We established this value as the upper limit on the basis of the radiographies of 350 newborn babies with clinically normal hips, who were followed for 12 months without any sign of C.H.D. or C.H.P. The same value was established as the upper normal limit in the radiographies of the hips of 55 still born babies in whom the dissection revealed normal hips.[10]

TREATMENT

All newborn babies with a positive Ortolani's sign were treated. The treatment of 18 babies was initiated between the third and sixth day after birth. The treatment of four babies in whom we detected a positive Ortolani's sign at birth, was started at 2 months of age (Figures 9, 10, 11, 12, 13, 14, 15, 16).

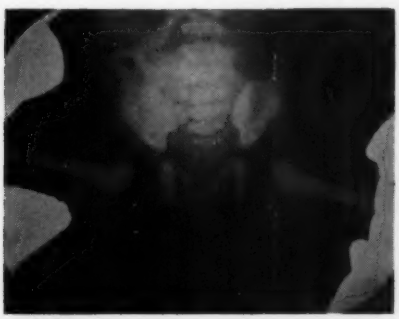

Figure 9 and 10

The radiography of the hips in antero-posterior and frog-leg view of newborn baby girl three days old shows acetabular angle over 28°. There was positive Ortolani's sign bilaterally. The treatment with Frejka pillow started at 2 months of age.

Figure 11 and 12

Radiographs of the hips of the same patient at 3 months of age. Clinically negative. Continued treatment with Frejka pillow.

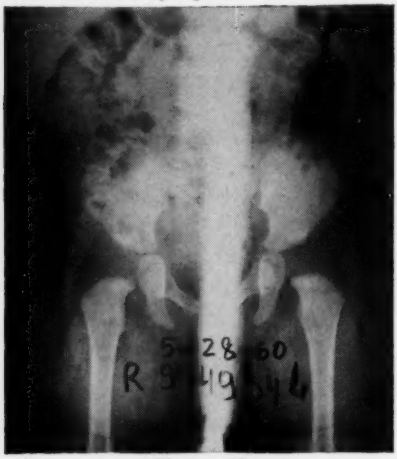

Figure 13

Radiography of the hips of the same patient at 5 months of age. Clinically negative. No appearance of the nucleus of ossification of the proximal femoral epiphysis. Treatment with Frejka pillow continued.

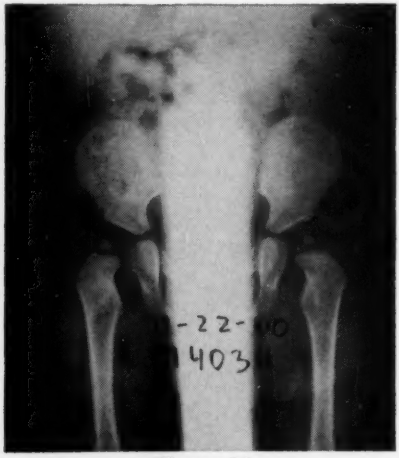

Figure 14

Radiography of the hips of the same patient at 11 months of age shows difference in size of the nucleus of ossification. Treatment discontinued. Discouraged the parents to allow the baby to stand or walk.

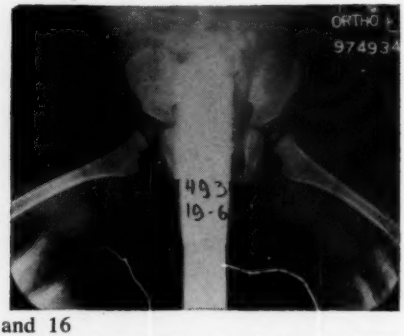

Figure 15 and 16

Radiographies of the hips of the same patient at 13 months of age. There is still very small difference in the size of nucleus of ossification, and the higher position of the left proximal epiphysis. The patient started to walk.

The treatment consisted of the application of the Frejka pillow (Figure 17)[5,6,11]. It is very important that the pillow is properly applied. The instructions given to the mother should be clear and followed by the demonstration of the Frejka pillow application.

Figure 17

Frejka pillow properly applied on the newborn baby.

The thighs have to be abducted foı 60-70° when the pillow is applied. The pillow should be applied over the diaper, and reapplied with the changing of diaper. In the beginning of the treatment the child has to be seen after two weeks for the control of the correct application of the Frejka pillow. After this examination the child is checked clinically and radiologically every 2½ to 3 months until the well developed acetabulum and well centered proximal femoral epiphysis radiographically and stable hip clinically are obtained.

In the 18 babies in whom the treatment started within a few days after birth, the hips were negative within 4-6 months.

Four cases in whom treatment started at two months of age had negative hips within 7-11 months.

The result of all treated children is excellent at the present time; however, many of them are still under periodic clinical control.

The changes in the Ortolani's sign in the newborn treated with the Frejka pillow were interesting. Two weeks after the beginning of treatment Ortolani's sign was still positive, but within 1-3 months disappeared completely.

It is important to emphasize that we had complete and excellent cooperation of the mothers in the cases whom we treated or in those whom we followed in the clinic for various above mentioned reasons. After the explanation of our work

on the hip problems and earliest diagnosis and treatment of the hip, we had both parents on our side.

CONCLUSIONS

1) From our three years experience we believe that the examination of the hips of the newborn babies by the Doctor who is acquainted with the exact technique, is of paramount importance in the diagnosis and earliest treatment of C.H.P. and C.H.D.

2) Since we started with the systematic examinations of the hips of all newborn babies in Henry Ford Hospital, we have not had a case of C.H.D. seen in our Orthopedic Clinic who was not diagnosed within a few days after birth.

3) Ortolani's sign is a reliable sign in the diagnosis of the C.H.P. in newborn babies.

4) All newborn babies with the positive Ortolani's sign had radiography with increased acetabular angle over 28°.

5) The treatment with Frejka pillow in the newborn with positive Ortolani's sign should be started within a few days after birth.

6) The hip examinations of the newborn babies, the clinical follow-up, the control of the position of the baby's hips while radiographies were taken, and the dissection of hips of the stillborn babies were all done personally by the author. In this manner all phases of the investigation were controlled by one person resulting in consistent findings.

SUMMARY

The Author examined personally 4300 newborn babies among whom 22 had a positive Ortolani's sign. The Author describes the technique of the examination of the hips, treatment and results.

Our investigations and examinations were possible only with the close and friendly help and cooperation of the Pediatric, Obstetric, and Radiology Departments.

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INTERESTING CASES OF CONGENITAL HIP DISLOCATION AND SUBLUXATION IN THE NEW BORN BABIES

STANKO STANISAVLJEVIC, M.D.*

In the period of 1958-1961 the author examined personally 4300 newborn babies among whom 22 had a positive Ortolani's sign.[4,5]

The cases described in this paper are included in the 4300 examined newborn babies, but not among the 22 cases in whom a positive Ortolani's sign was detected.

CASE PRESENTATION

H. G. The newborn baby girl delivered spontaneously in occiput position was the fifth child. There was no history of congenital hip dislocation (C.H.D.) in the families of her father (who is of German descent) and her mother (who is of Italian parentage).

This baby's hips were examined on the second day after birth. Asymmetry of the skin folds was not noted. Ortolani's sign and abduction of the thighs were negative; however, extreme looseness of both hips was detected. Telescoping of both hips in antero-posterior and infero-superior directions was possible without the feeling of any resistance. Both hips could be dislocated by the simple motion of the baby's body, while the lower extremities were held. The hips were relatively stable only in the frog leg position with the thighs abducted at 75-85°. Good radiographies were very difficult to obtain, because of the difficulty of keeping the baby quiet. Every motion of the body would produce dislocation of the hips. The radiographies showed increased acetabular angle[7] above 28°, and the push-pull radiographies of both hips showed clearly a dislocated hip on the pushed side.

Treatment started with the application of the Frejka pillow on the third day after birth. Each day while in the hospital nursery the baby's hips were examined with the applied Frejka pillow and always found to be dislocated. On the seventh day after birth, the bilateral plaster hip spica was applied with the hips in the frog leg position abducted at 80°. The child had to be sedated during the plaster cast application, otherwise the hips could not be kept well reduced. The plaster cast did not cause any visible discomfort or inconvenience to the baby and was removed 3 months after its application. At that time clinical examination of the hips was negative. Radiographies showed considerable degree of antiversion of the proximal part of the femur bilaterally. The Frejka pillow was applied and kept for two months after which time clinical examination of both hips continued to be negative. Radiography showed well developed acetabuli, with great amount of antiversion of the proximal femoral end. (Figures 1-9).

SECOND CASE

This case is one of a group of 5 newborn babies in whom the author detected an interesting clinical sign. This group of newborn babies was examined

*Department of Orthopedic Surgery, Orthopedic Research Laboratory.

Figure 1 and 2

H. G. 2 days old. Radiographies of the hips in antero-posterior and frog leg position show an increase of acetabular angle.

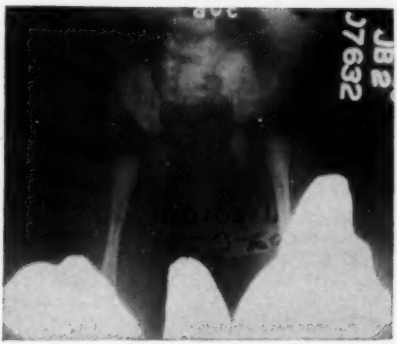


Figure 3

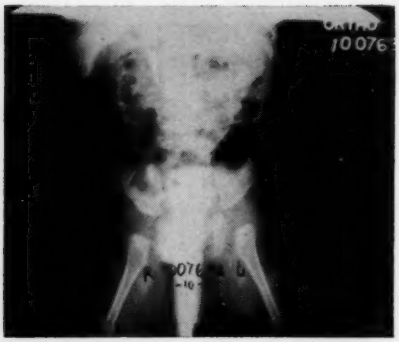
Radiography of the same patient in whom the left hip was pushed cranialward and the right hip was pulled caudalward. Note complete dislocation of the left hip.

Figure 4

Radiography of the same patient in whom the right hip was pushed cranialward and the left hip was pulled caudalward. Note complete dislocation of the right hip.

Figure 5

Radiography of the same patient in whom both hips were pushed cranialward. Note dislocation of both hips.



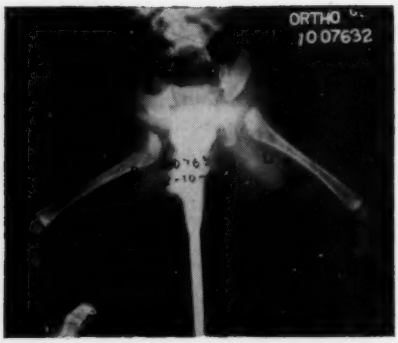
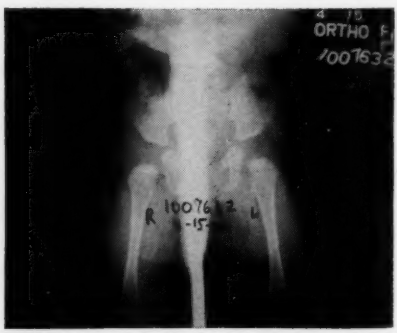


Figure 6 and 7

Radiographies of the same patient in antero-posterior and frog leg position views at 3 months of age when the plaster spica was removed. Note the conspicuous amount of antiversion of both proximal ends of the femur. Treatment with the Frejka pillow began.



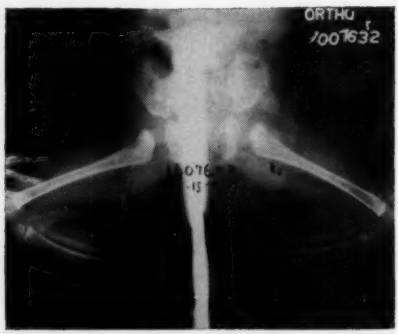


Figure 8 and 9

Radiographies of the same patient in antero-posterior and frog leg position views at 5 months of age. Note well developed acetabuli and great amount of antiversion of the proximal femoral ends bilaterally.

with the usual technique[4,5] for Ortolain's sign. During the examination, the newborn baby was supine on the examining table with the thighs flexed at 90°. The author's hand exerted moderate pressure on the knee downward to transmit pressure to the femur and hip. At that moment the examiner had the feeling of a smooth elastic-like motion of the femoral head rather than the sign of "click". Keeping the same amount of pressure the examiner abducted the thigh to 85°. In that phase of the examination neither resistance nor "click" were felt. The same sign was detected bilaterally in the 5 newborn babies, who were followed by the author in the Pediatric and Orthopedic Clinics.

P.M. was a newborn baby girl delivered spontaneously in the occiput position, the second child of a Polish family. On the second day after birth the author examined the baby's hips and detected the above described sign bilaterally. Radiography was done and showed increased acetabular angle above 28°. (Figure 10, 11). The author was informed by the mother that there was no history of C.H.D. in her

Figure 10 and 11

P.M. Newborn baby girl 2 days old. Radiographies of the hips in antero-posterior and frog leg position views show increased acetabular angle bilaterally.

or her husband's family. Their first daughter, 1½ years of age, was wearing an abduction splint for congenital subluxation of the hips (C.H.S.) bilaterally, and in treatment elsewhere. At one year of age C.H.S. was discovered accidentally on radiography of the pelvis for kidney and bladder stones in another hospital.

Our patient was re-examined at two months of age, at which time the hips were negative clinically, but the radiographies still showed an increased acetabular angle. (Figures 12, 13). Treatment of the baby's hips was not begun yet.

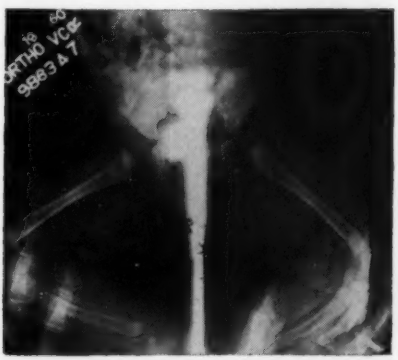


Figure 12 and 13

Radiographies of the hips of the same patient at 2 months of age. Antero-posterior and frog leg position views still show increased acetabular angle.

At 4 months of age the hips were negative clinically. The radiographies still disclosed an increased acetabular angle and the beginning of subluxation of both hips. (Figures 14, 15). The patient was not examined for the next 6 months, because the parents failed to keep appointments.

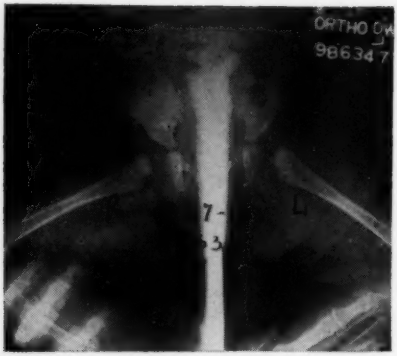
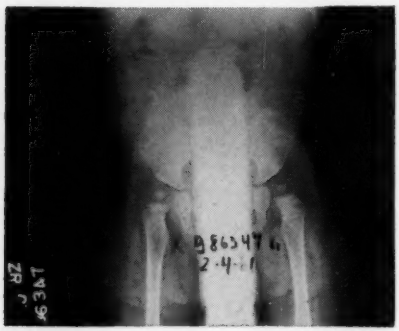


Figure 14 and 15

Radiographies of the hips of the same patient at 4 months of age still show increased acetabular angle and the beginning of the hip subluxation bilaterally.



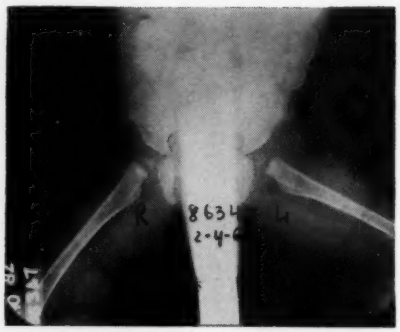


Figure 16 and 17

Radiographies of the hips of the same patient at 10 months of age show subluxation of both hips.

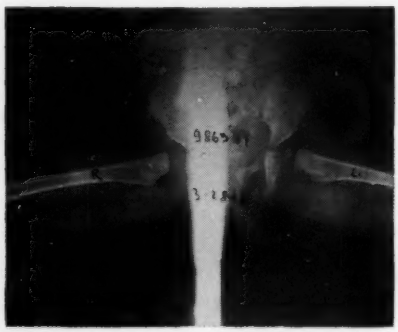


Figure 18 and 19

Radiographies of the hips of the same patient at 12 months of age show increased degree of subluxation of the hips bilaterally.

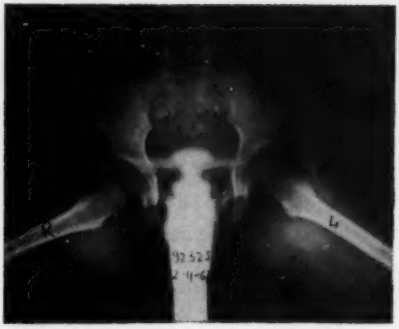


Figure 20 and 21

Radiographies of the hips of our patient's sister at 2½ years of age show bilateral subluxation of the hips.

At 10 months of age the hips were negative clinically, and the radiographies showed subluxation of both hips. (Figures 16, 17). The patient was not treated. At the request of the parents the author examined the older daughter, now 2½ years of age, and still in treatment elsewhere with abduction apparatus for C.H.S. The hips were negative clinically and the radiographies showed C.H.S. bilaterally.

Our patient was examined for the last time at 12 months of age, at which time the hips were negative clinically, and the radiographies revealed increased subluxation in both hips. (Figures 18-21). The patient had not begun to walk. The author recommended the beginning of the treatment with abduction apparatus.

DISCUSSION

The author believes that all these cases deserve full attention. In the first case Ortolani's sign and limitation of abduction of the thighs were negative. The author thinks that the reason the hips dislocate so easily is a consequence of a very shallow acetabulum with well rounded ridges, enlarged articular capsule, long or absent ligamentum teres and deformed femoral head. The clinical picture of this case is not similar to C.H.D. known as atypical[3], teratologic[2,8] or embrgologic[1]. We believe that in our case the best way of treatment was the application of the plaster hip spica in frog leg position. The application of the Frejka pillow as initial treatment was shown to be inadequate and dangerous, because of fixation of both hips in the dislocted position. In similar cases the plaster hip spica should be applied very shortly after birth (perhaps on the second or third day), to avoid trauma of the easily dislocating hips. Having only one case of this kind of C.H.D. among 4300 newborn babies, we believe it to be rare.

We have no experience with a similar case of C.H.D. detected at birth with the same clinical and radiological signs and not treated; however, we assume that delayed treatment would not give good results in so short a time.

The second group is characterized by the sign which predicted the development of C.H.S. We were unable to find it described in the literature. We do not know

the pathology of this sign which was present at birth and disappeared within the first two months.

We detected this sign in 5 newborn babies 3 of whom developed subluxation of both hips of various degrees, depending upon the age of the patient. The oldest one is the case described here. However, all cases are under regular clinical control.

A comparison of the clinical and radiological signs in the hips of our patient and those of her older sister are extremely interesting. It is our prediction that the hips of our patient will produce the same picture as those of her sister.

CONCLUSIONS

1) The described case of C.H.D. is very rare showing a ratio of 4300:1.

2) In such a case the diagnosis within a few days after birth and immediate treatment with the plaster hip spica is of paramount importance.

3) The examiner should be alert and search for the sign which could predict C.H.S. and evaluate it when found.

4) The variety of signs by which congenital hip pathology could be detected[4,5,6] shows that the hip examination of the newborn babies is justified and requires a certain manual skill as well as complete knowledge of all signs.

SUMMARY

Six cases, one of C.H.D. and 5 with an interesting sign detected in the hips, found during the examination of 4300 newborn babies by the author, are described.

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BIO-SCIENCE SEMINAR—CHROMOSOMAL DISORDERS IN MAN*

RICHMOND W. SMITH, JR., M.D., Presiding

Subject: Chromosomal Disorders in Man

Moderator: JOEL I. HAMBURGER, M.D.

Discussants: MARTIN Z. FRUCHTMAN, M.D.

RAYMOND GUEVIN, M.D.

DR. HAMBURGER: The study of chromosomal disorders in man has been given great impetus by the recent development of two techniques: the buccal smear for sex chromatin, and direct study of human chromosomes. As a result of these developments, a multitude of reports have been submitted defining the chromosomal complements of numerous disorders of sexual and/or somatic differentiation. Understanding these disorders and the underlying hereditary mechanisms requires familiarity with some of the basic principles and terminology of modern cytogenetics. It is our purpose this evening to review this material. Dr. Fruchtman would you please define and compare the phenomena of meiosis and mitosis, and review for us the mechanism of sex differentiation?

DR. FRUCHTMAN: Meiosis is the process by which mature reproductive cells or gametes are produced (Fig. 1). In this process two major divisions take place, the first of which is reductional and the second, the equation division.

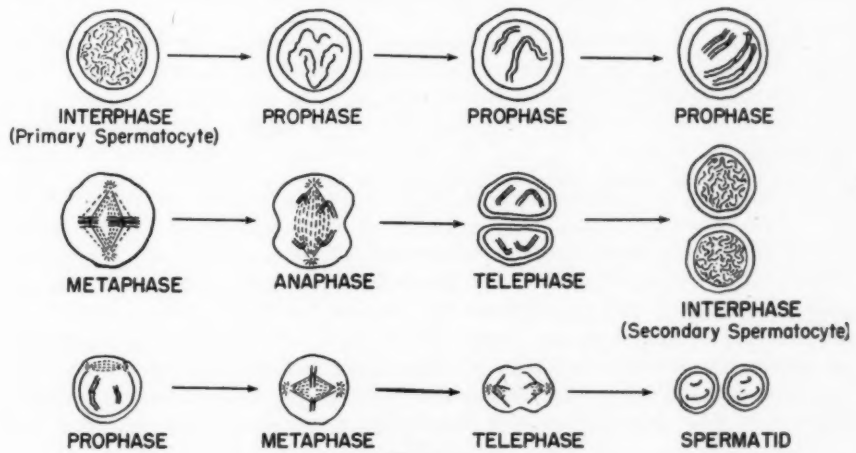


Figure 1
Meiosis.

In the initial phases of meiosis the immature reproductive cell, spermatogonium or oögonium, enlarges to become a primary spermatocyte or oöcyte. Next, the diffuse chromatin material within the resting nucleus condenses to form distinct chromo-

*This is an edited report of the Bio-Science Seminar presented by the Division of Endocrinology at the Henry Ford Hospital February 6, 1961.

somes, which in turn pair off and line up on the spindle fibers prior to separating into the two daughter cells, called secondary spermatocytes or oöcytes. In the case of the oöcyte one daughter cell preempts most of the cytoplasm, and its tiny sister cell is left as a polar body. Note that in late prophase each single chromosome consists of paired identical chromatids joined by a centromere or kinetochore. This first meiotic division is referred to as the reduction division because the resultant cells have one-half the original number of chromosomes. In the example of Figure 1 the full chromosomal complement is four. This is called the diploid number of chromosomes; the haploid number is two.

The second meiotic division is simply an example of mitosis, or the sequence of nuclear and cytoplasmic events by which a cell divides into two identical off-springs, each of which, in contrast to the products of the first meiotic division, is an exact replica of the mother cell. In the mitotic or equation division the diffuse chromatin of the interphase nucleus again forms distinct chromosomes in prophase. The chromosomes are split longitudinally into paired chromatids which line up on the spindle fibers. In anaphase a chromatid from each pair separates to opposite poles of the dividing cell to become a chromosome in its own right. In telephase the nuclear membrance envelopes the chromosomes and the cytoplasm divides producing two daughter cells each of which contain the same number of chromosomes as the mother cell. These cells are called spermatids or, in the female, ovum with its polar body. In the further maturation of the spermatids into spermatozoa the cytoplasm is drawn out into a tail leaving the head consisting primarily of the nucleus.

The union of sperm and ovum, forming a zygote, restores the diploid number of chromosomes. At the moment of fertilization genetic sex is established as seen in Figure 2. Females have two X chromosomes, whereas males have an X and a Y. As a consequence of meiosis all ova normally have a single X chromosome, while spermatozoa have either an X or a Y. Fertilization of an ovum by an X sperm produces a genetic female, whereas fertilization by a Y sperm, of course, produces a male.

In man the full chromosomal complement or diploid number is 46, 22 pairs or 44 non-sex chromosomes (autosomes) and two sex chromosomes.[2] The latter are paired X's in the female and unpaired X and Y in the male. Under unusual circumstances some individuals have chromosomal counts which differ from the normal or euploid state. Such a condition is called aneuploidy or heteroploidy, and if a simple multiple of the haploid chromosomal count exists it is termed polyploidy, examples of which are triploidy with 69, or tetraploidy with 92 chromosomes.

DR. HAMBURGER: Dr. Guevin, would you please explain for us the process by which the indifferent fetus, equipped with primordia of both sexes, develops into either a male or female individual?

DR. GUEVIN: Sex differentiation can be divided into three distinct phases, the first of which is gonadal differentiation beginning at the sixth week of intrauterine life. At this time the primitive gonad with both cortical (ovarian) and medullary (testicular) primordia, starts along the path to specific sexual orientation,

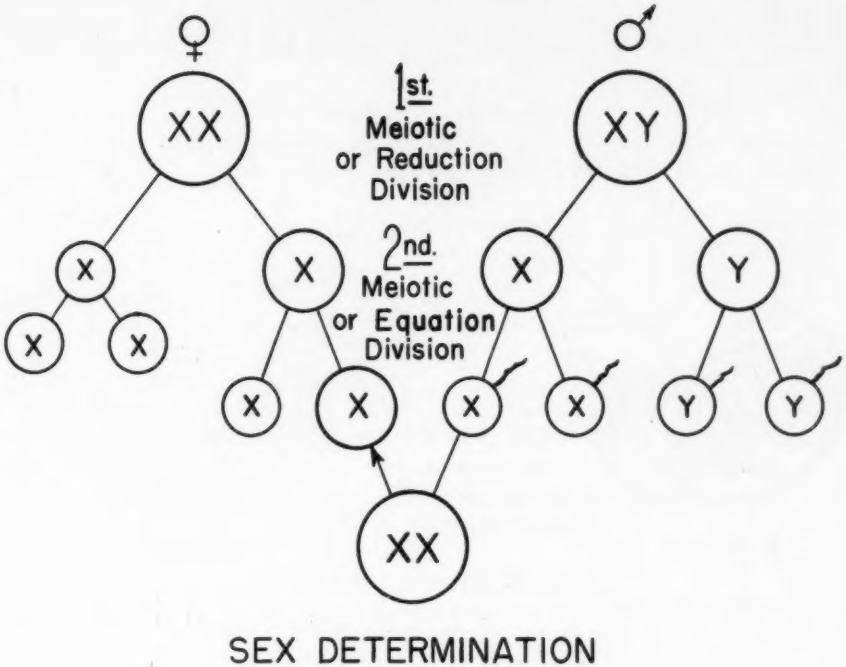


Figure 2
Sex determination.

influenced by the balance of genetic factors established at the moment of fertilization (Fig. 3). In the case of XY genetic sex, medullary structures predominate and a testis will form, whereas in the XX individual, cortical structures develop into an ovary. The next phase, that of differentiation of the internal genitalia or genital ducts (Fig. 4), is postulated to be under the control of "evocators" produced by the developing gonad. These evocators are not hormones in the usual sense since their action is local and unilateral, as evidenced by the situation in the hermaphrodite with an ovary on one side, a contralateral testis, and genital ducts appropriate to the respective gonads. Similarly, Jost[6] has shown that in rabbits castrated before there has been any gonadal differentiation, a foetal testis grafted unilaterally will lead to the development of male internal genital ducts on that side. The third phase in sexual differentiation is the development of the external genitalia, and it is important to recognize that this step is entirely hormonally controlled. In contrast to the development of internal genital ducts, the final configuration of the external genitalia can be influenced by sex hormones, whether from gonadal, adrenal or exogenous sources (Figs. 5, 6).

The full significance of the foetal gonad influence on sex differentiation has greatly been clarified by Jost[6] who showed that foetal rabbits castrated prior to gonadal differentiation always develop as females. However, internal and external genitalia remain rudimentary (Fig. 4). The development of the male phenotype

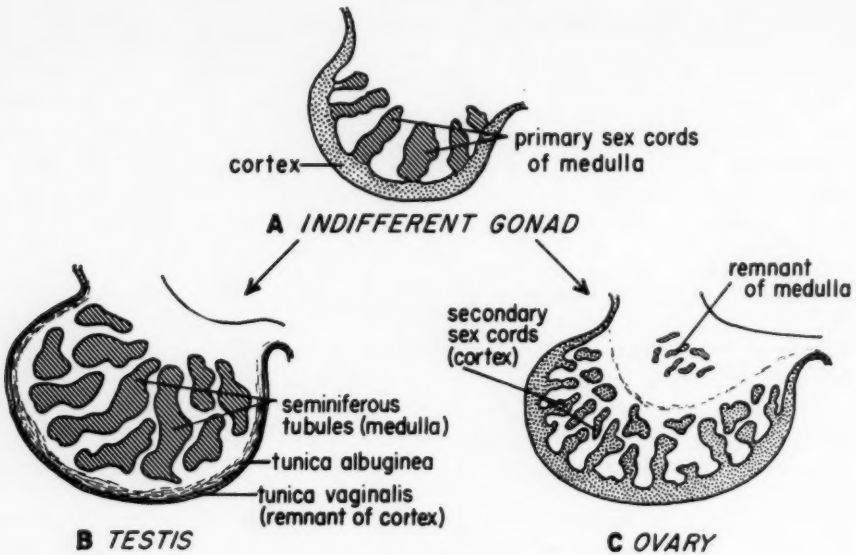


Figure 3

Differentiation of the indifferent gonad (from Barr[44], by permission of the author).

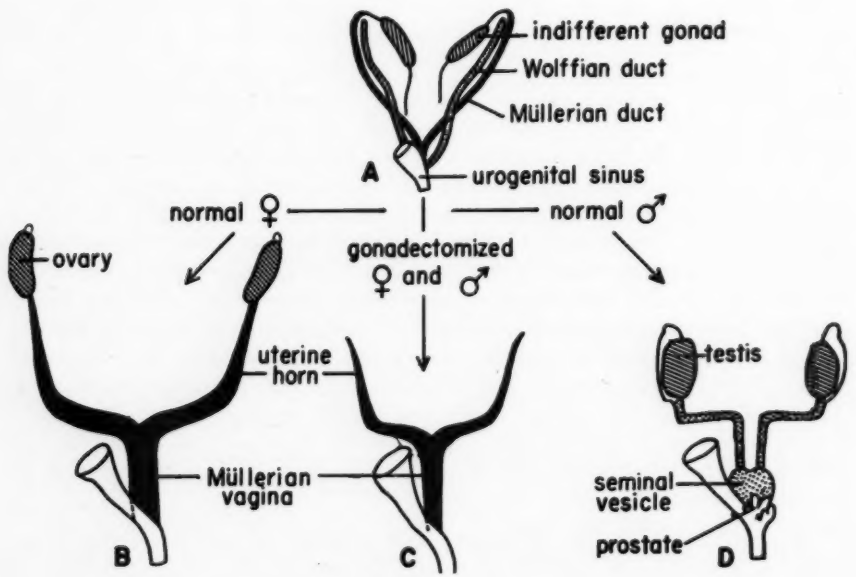


Figure 4

Differentiation of genital ducts (from Jost,[45] by courtesy Cambridge University Press).

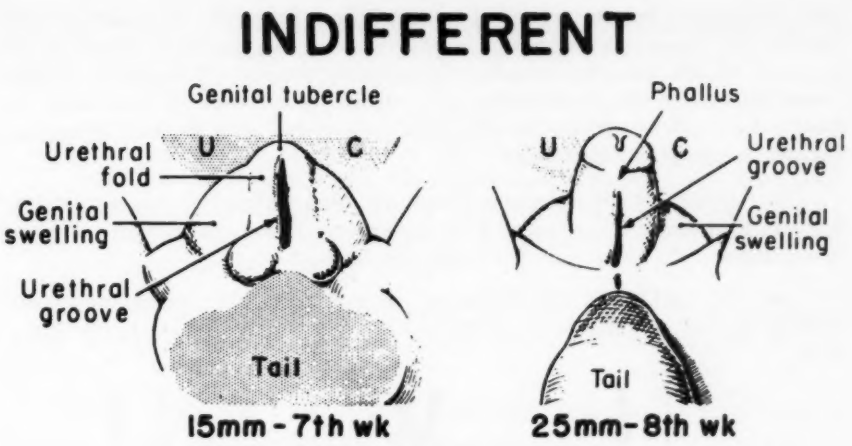


Figure 5
Differentiation of external genitalia (as depicted in Astwood[3]).

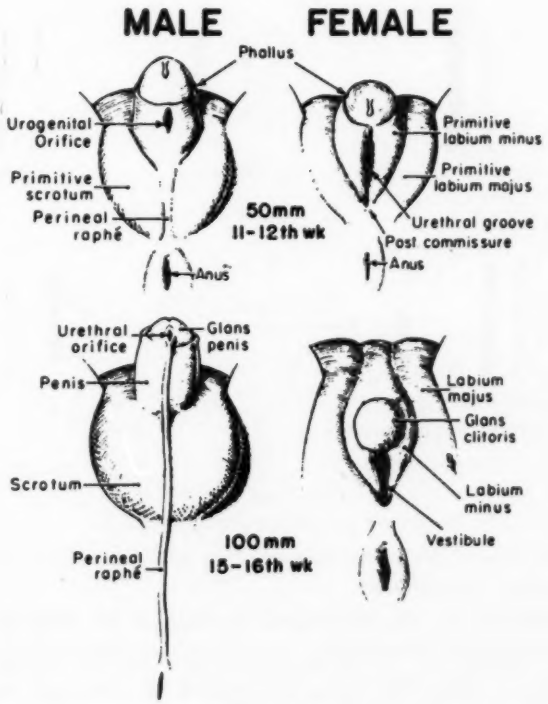


Figure 6
Differentiation of external genitalia cont. (as depicted in Astwood[3]).

thus requires the presence of specific masculinizing substances of gonadal origin, whereas the female phenotype can develop without such mediators, although sex structures will be somewhat immature. In summary, gonadal differentiation is genetically determined; differentiation of the internal genital ducts is determined by specific local evocator substances of gonadal origin; and differentiation of external genitalia is hormonally determined.

DR. HAMBURGER: Departures from the usual sequence of events in meiosis can lead to defective transmission of the genetic complement to the zygote, resulting in a mutation. Dr. Fruchtman, how might this happen?

DR. FRUCHTMAN: The important mechanisms include crossing over, translocation, deletion, inversion and non-disjunction (Fig. 7). We might briefly define these:

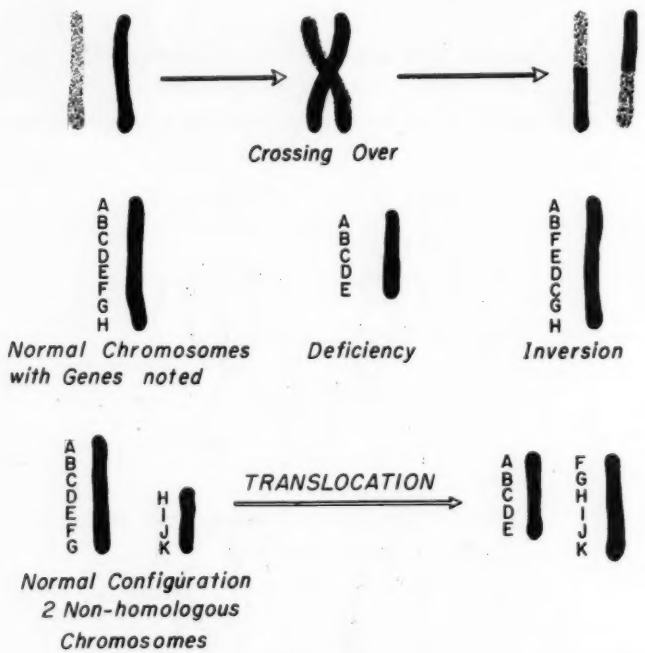

Crossing Over
A B C D E F G H
Normal Chromosomes with Genes noted
A B C D E
Deficiency
A B F E D C G H
Inversion
A B C D E F G
H I J K
TRANSLOCATION
A B C D E
F G H I J K
Normal Configuration 2 Non-homologous Chromosomes

Figure 7.
Mechanisms of aberrant genetic transmission.

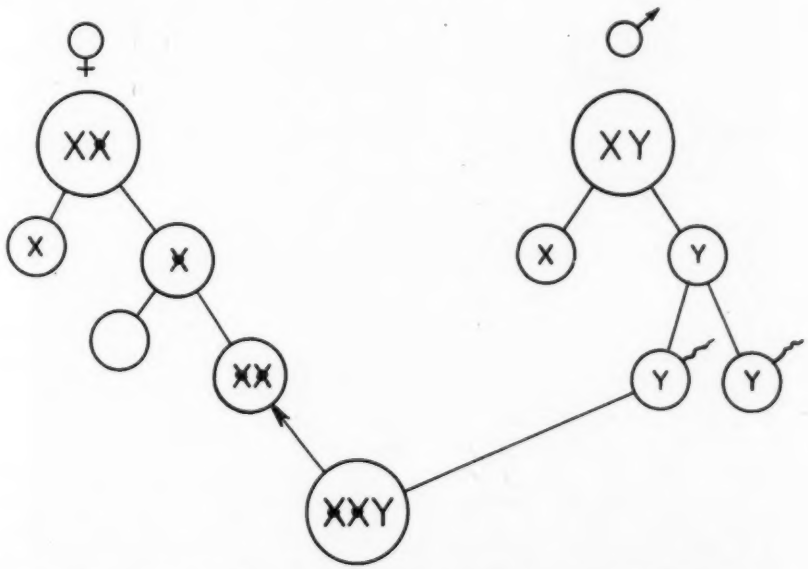
1. Crossing over refers to the exchange of chromatin material between adjacent homologous chromosomes.
2. Translocation is a similar process but involves an exchange of chromatin material between chromosomes which are *not* paired or homologous.
3. Deletion is simply the loss of a segment of chromatin material from a chromosome, leaving the cell deficient in the genetic influence carried on that chromatin segment.

4. Inversion involves only a change in the order of genetic determinants on a given chromosome — none having been added or substracted.

5. Non-disjunction differs from the above processes in that entire chromosomes may be gained or lost as cells divide. As shown in Figure 8, the X chromosomes fail to separate at the second meiotic division so that the mature ovum contains both X chromosomes, while its polar body has neither. If such an ovum is fertilized by a Y sperm (as in this example) an XXY individual results. This, of course, is the sequence of events which lead to the anomalies of Klinefelter's syndrome.

The environmental factors which favor the occurrence of these mechanisms include increased temperature, radiation, drugs (e.g. colchicine) and advanced maternal age.[1]

DR. HAMBURGER: Since many chromosomal disorders in man result in abnormal sexual differentiation, the buccal smear technique for determining sex chromatin, a rapid method of screening for genetic sex, was hailed as a significant contribution to a better understanding of these disorders. Dr. Fruchtman, how are such smears prepared, and can you show us an example?



Transmission of color blindness in Klinefelter's Syndrome when neither parent is color blind

Figure 8

Transmission of color blindness in Klinefelter's syndrome when neither parent is color blind. (the X with the central dot carries the gene for color blindness).

DR. FRUCHTMAN: Buccal smears are prepared by gently scraping the buccal mucosa with a tongue blade and transferring the epithelial cells to a glass slide. The smear is fixed immediately in 95% alcohol, or in equal parts 95% alcohol and ether, and later stained with a basic dye. Some laboratories employ mild acid hydrolysis to accentuate the sex chromatin. Males are considered chromatin negative since less than 6 per cent of their nuclei contain sex chromatin. Females are normally chromatin positive since 40 to 80 per cent of their nuclei contain sex chromatin.

DR. HAMBURGER: Dr. Guevin, what does the sex chromatin mass represent?

DR. GUEVIN: In 1949 Barr first called attention to the significance of the clump of chromatin material found at the inner border of the nuclear membrane of the resting cell (Fig 9). This structure was regularly observed in cells of female origin but uncommonly, if at all, in those of male. This is the so-called sex chromatin mass, or chromatin bar. It measures about one micron in diameter and is composed of deoxyribonucleic acid. This mass of nuclear chromatin, clumped and heterochromatic (or heteropycnotic), is stainable and visible in the intermitotic nucleus, whereas the rest of the nuclear chromatin material becomes dispersed.

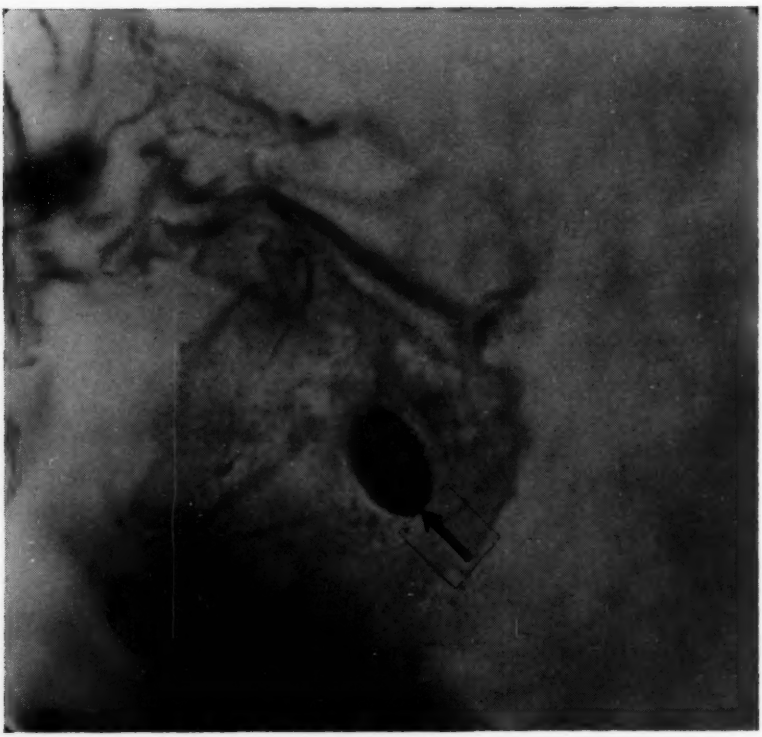


Figure 9
The sex chromatin mass.

In spite of Barr's recommendations of caution regarding the significance of his finding, the impression that nuclear sex is equivalent to genetic sex gained widespread acceptance.

In addition, it was accepted that in the female phenotype the chromatin bar represented fused portions of two X chromosomes, and that its absence in the male was due to the fact that only a single X chromosome is present. This reasoning is compatible with the observations both in chromatin-negative Turner's syndrome where only a single X chromosome is found, and in chromatin-positive Klinefelter's individuals with two X chromosomes. However, earlier studies in chickens, silkworms and some insects revealed that females with only one sex chromosome were chromatin positive.[8,36] Furthermore, in human studies two chromatin bars have been found in the XXX[9] and three in the XXXX females.[10] These observations seem to indicate that a single X chromosome can produce a sex chromatin mass, and that the number of such masses found is one less than the number of X chromosomes in the nucleus. Ohno[11] suggested that the heterochromatic X chromosomes, i.e. those which produce chromatin bars, are of paternal origin and that the maternal X remains dispersed (euchromatic or isopycnotic). However, in chromatin-positive Klinefelter's syndrome there is evidence that both X chromosomes are derived from the mother. but one chromatin bar is seen. If the X chromosome of paternal origin were always heterochromatic one would expect one-half of females to have two chromatin bars -- one representing the X chromosome from the father and the other to have been derived from the materal grandfather.

An alternate viewpoint is that all X chromosomes are heterochromatic but that one chromosome has become dispersed to satisfy the metabolic and genetic requirements of the resting cell.[12] All additional X chromosomes would then remain in the heterochromatic state. Although having the virtue of simplicity, this exposition fails to account for the tetraploid state in chromatin-negative individuals with 92 chromosomes, including two X's and two Y's.[13]

An explanation which would cover all of the above situations assumes that each set of 44 autosomes can suppress heterochromaticity of one X chromosome.[13] There still remains, however, the recently described patient with Turner's syndrome with an XO chromosomal complement and a chromatin positive buccal smear. Perhaps the XO classification was an error of interpretation since it is well known that the X chromosome cannot positively be distinguished from autosomes of pairs six to twelve.[10,14] Hence, possibly two X chromosomes were present and one autosome was missing. An alternate view would involve translocation of the heterochromatic portion of the missing X chromosome to one of the autosomes, with deletion of the remainder of the X chromosome. In this particular research area it seems that exceptions have managed to remain one step ahead of explanations.

DR. HAMBURGER: Although the buccal smear is a valuable screening tool, definitive evaluation of chromosomal disorders, whether of sex chromosomes or of autosomes, necessitates actual chromosomal counting. Dr. Fruchtman, in general how is this done?

DR. FRUCHTMAN: In the preparation of cells for chromosomal counts, bone marrow is usually used, but skin and gonadal tissue, and leucocytes from peripheral blood have also been employed. Cells are cultured for five hours, and during the last two hours colchicine is introduced into the medium to arrest mitosis at metaphase. The cell culture is then treated for 10 minutes with a hypotonic solution causing the cells to swell and the chromosomes to disperse. The preparation is fixed, stained and squashed between a glass slide and coverslip to spread the chromosomes which may then be observed in one optical plane under the oil immersion objective. Photomicrographs are taken; the chromosomes are cut out and paired according to centromere position, arm lengths and ratios of their parts. The result is the karyogram or idiogram shown in Figure 10. One employs the term karyotype to refer to a specific chromosomal makeup, e.g., one with Klinefelter's syndrome might have an XXY karyotype. This should be differentiated from genotype which indicates the specific gene complement, and phenotype which refers to physical configuration.

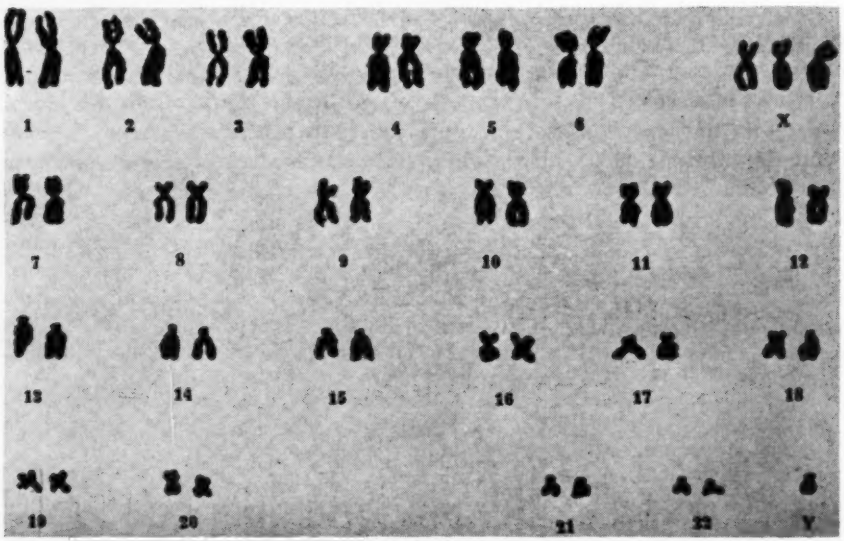


Figure 10
Karyogram of XXXY Klinefelter's individual (from Ferguson-Smith[36]).

DR. HAMBURGER: Dr. Fruchtman, what are some of the criticisms of this technique?

DR. FRUCHTMAN: The assumption that chromosomes of similar morphology are in fact homologus is fraught with danger. It is a fundamental principle of genetics that proof of the homologous nature of two chromosomes depends upon the demonstration of pairing during mitosis.[1]

Because of defects in the technical aspects of the method, it is unusual even in normal individuals for every metaphase cell to contain the same number of

chromosomes. Hence, in actual practice the range of chromosomal numbers is determined. The majority of atypical cells usually have less than the modal (or most common) number, having lost one or more chromosomes in the process of preparation. At times, however, cells are found with an extra chromosome which has been spuriously introduced. Nevertheless, the modal number usually predominates when enough properly prepared cells are counted. It is important not to include cells in the count when: (1), the chromosomes are poorly spread; (2), the cells are ruptured; or (3), one or more chromosomes are split at their centromeres into sister chromatids. It is generally assumed that counts differing from the modal count are artefacts, but they could represent either abnormalities during mitosis or mosaics. In a recent report Berman[15] has suggested the direct examination of marrow cells, avoiding incubation, as a means of reducing the artefact inherent in the method.

DR. HAMBURGER: Dr. Fruchtman, what is mosaicism and what criteria must be established to confirm the existence of a mosaic?

DR. FRUCHTMAN: Mosaic is a term applied to an individual in whom cells exist with more than one chromosomal number. For a mosaic to result it is apparent that the anomalous events must occur after fertilization of the ovum and formation of the single cell zygote. Any defect in maturation of germ cells would be reflected in the zygote itself and, hence, in all cells of the developing embryo. Furthermore, such an event must occur soon after the zygote is formed — probably in the first or second division — if the final individual is to have any significant number of atypical cells. As noted by Ford[16] the proportion of cells deviating from the species number may not be the exact fraction predicted on the basis of the time at which

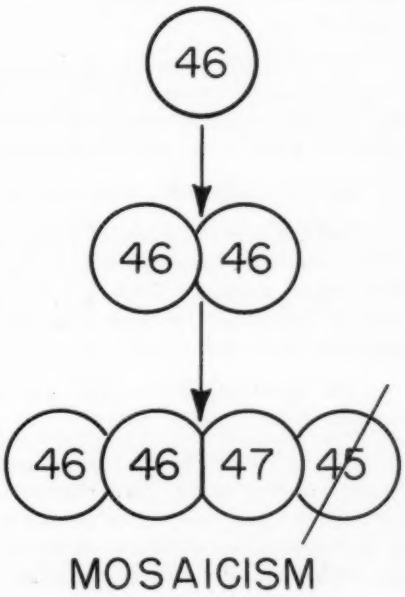


Figure 11

Mosaicism.

the anomalous mitotic division took place, since selective forces appear to operate which favor the survival and multiplication of cells with the more nearly normal chromosomal complement. For example, (Fig. 11) if the defect occurred at the second mitotic division of the zygote the result would be two normal and two abnormal cells. Neither abnormal cell might be viable, but if one survived and continued to divide, one might expect that the final individual would be a mosaic of two-third normal and one-third abnormal cells. However, as a result of unfavorable selective influences acting upon the abnormal cell the final mosaic ratio might actually be three to one. The degree of selectivity also could vary from organ to organ such that in marrow the percentage of abnormal cells might be 30, while in the skin 5 or less.

Since it is unusual for all metaphase cells to contain the same number of chromosomes even in normal individuals, Court-Brown[17] has suggested criteria which must be satisfied in the establishment of a mosaic:

(1) The number of cells with counts differing from the modal number must be greater than reasonably may be attributed to chance, usually about 30 per cent.

(2) This excess of atypical cells must be found in two or more cell cultures, preferably in two or more tissues.

(3) A high proportion of atypical cells should have the same karyotype, that is, the same chromosomes are deficient or in excess.

DR. HAMBURGER: Of the chromosomal disorders, the first to be described was the trisomy of chromosome No. 21 in mongolism. Dr. Guevin, how is this explained?

DR. GUEVIN: The trisomy of mongolism is presumed to result from maternal nondisjunction of chromosome No. 21 during oögenesis, producing an ovum with two such chromosomes. When such an ovum is fertilized by a normal sperm, trisomy results, or three of the No. 21 chromosome.[18]

DR. HAMBURGER: What is the karyotype of parents and offsprings of mongols?

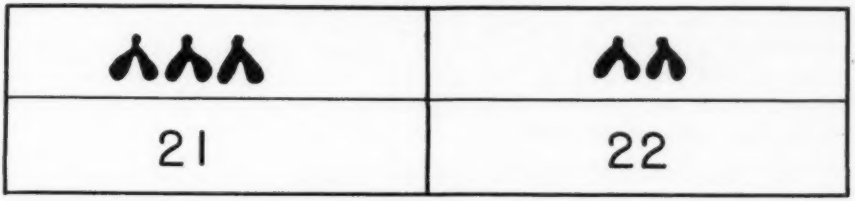
DR. GUEVIN: Parents of mongols usually have a normal karyotype, but one father (with an apparently normal phenotype) has been reported with trisomy of chromosome nineteen.[20] This will be discussed later. When mongols are fertile, offspring are usually mongols; however, in some cases a normal karyotype and phenotype have been found.[19]

DR. HAMBURGER: How does familial mongolism differ from the more frequent sporadic cases?

DR. GUEVIN: Familial mongolism with a chromosomal count of 46 has been reported without an advanced maternal age factor. However, close study of some cells revealed that one of the autosomes of pair 22 was larger than its mate and an additional minute chromatin particle was present (Figure 12). This was apparently the result of translocation of the greater part of a third No. 21 chromosome to a

No. 22, with the extra chromatin particle representing the residual centric fragment of that third No. 21 chromosome. Hence, the result is essentially trisomy of the 21st chromosome.[21]

SPORADIC MONGOLISM



FAMILIAL MONGOLISM

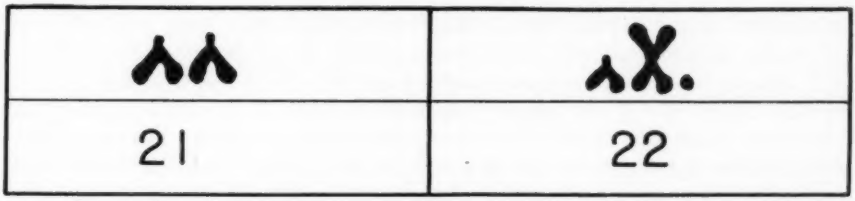


Figure 12

Familial mongolism (numbers below chromosomes indicate their idiogram position — see Figure 10).

	PATIENT	MOTHER MAT. AUNT MAT. G. M.	FATHER BROTHER
15	^	^	^ ^
21	^ ^	^	^ ^
15/21	X	X	
No. Chromosomes	46	45	46

Figure 13

Familial mongolism, alternate type (figures in column to the left indicate pair number of chromosomes depicted).

In another study a mongol boy was found to have 46 chromosomes while his mother, maternal aunt and maternal grandmother each had 45. Analyses of the idiograms revealed that the father and brother had normal chromosomal complements of 46. The boy, although having 46 chromosomes, had only one of pair 15 and an extra chromosome with the configuration of those in pairs six to twelve. The mother, maternal aunt and maternal grandmother had only one chromosome of pairs 15 and 21, but they also had the extra chromosome described above (Fig. 13). The interpretation is that the extra chromosome represents a fusion of one chromosome of pair 15 with one of pair 21. This results in a normal genetic complement in the female relatives, and provides for the equivalent of 47 chromosomes with trisomy of No. 21 for the mongol son.[43]

DR. HAMBURGER: Another example of trisomy is the XXY karyotype in Klinefelter's syndrome. Dr. Fruchtman, what is the evidence for maternal non-disjunction in this disorder?

DR. FRUCHTMAN: Non-disjunction during oögenesis was suggested by two observations. The first was that a mean maternal age of 33 years was observed for patients with this disorder in a population with an overall mean maternal age of 27.5 years.[22] Mental retardation is more frequent in the progeny of mothers with relatively advanced age, and mental retardation occurs with increased frequency in Klinefelter's syndrome. Since it is known that advanced maternal age predisposes to non-disjunction, the above has been considered suggestive evidence. The second observation, of greater genetic value, related to the occurrence of color-blindness in patients with Klinefelter's syndrome where both parents had normal color vision. Color vision is a sex-linked recessive trait carried on the X chromosome, and had the father been responsible for the patient's color-blindness he too should have been color-blind, since there is no corresponding gene on the Y chromosome to oppose the expression of this trait. Since neither the father nor the mother were color-blind, it must be presumed that the mother was a heterozygous carrier of the trait. Furthermore, as shown in Figure 8, non-disjunction can be pinpointed to the second division of meiosis, since this is the only time at which a gamete could be produced[22] with two chromosomes carrying genes for color-blindness.

DR. HAMBURGER: Dr. Fruchtman, what other karyotypes have been observed with the picture of Klinefelter's syndrome?

DR. FRUCHTMAN: Examples of Klinefelter's syndrome have now been reported with karyotypes of XX, XXY, XY,[22] XXYY,[23] XXXY,[24] and a mosaic of XXY/XX.[25] These are explained on the basis of various combinations of non-disjunction and translocation.

DR. HAMBURGER: Klinefelter's syndrome and mongolism would appear to have much in common from the genetic standpoint. Dr. Fruchtman, would you summarize these similarities for us?

DR. FRUCHTMAN: Mongolism and Klinefelter's syndrome have the following in common: (1), they result from non-disjunction during oogenesis; (2), there is a relatively advanced maternal age; and (3), mental deficiency, the rule in mongolism,

occurs with increased frequency in Klinefelter's syndrome. As might be expected in disorders with common etiologic factors, it was not long before individuals were reported with both disorders and 48 chromosomes.[26,27] Also reported was a case with 49 chromosomes with trisomy at two autosomal positions;[25] and instances of triploidy[28] and even tetraploidy[13] have been seen. These observations indicate that non-disjunction can involve any or all chromosomal pairs in single or multiple fashion.

DR. HAMBURGER: A more recently described example of trisomy is the so-called "super-female". Dr. Guevin, could you describe these women?

DR. GUEVIN: These inappropriately named women with an XXX karyotype are usually underdeveloped sexually, cease menstruating at an early age, and are infertile. However, two fertile cases have been described and in both the offsprings were apparently normal males.[9,29,30]

DR. HAMBURGER: Dr. Guevin, what other instances of trisomy have been described and what is their significance?

DR. GUEVIN: Trisomy has been reported with chromosomes 15, 17, 19 and 22. In most cases there were associated congenital anomalies, but the trisomy of No. 19 chromosome was found in an apparently normal male who was studied only because he fathered a mongol. These reports suggest that trisomy may be found more frequently than might be expected, and since it can occur without overt evidence of abnormality, the mere association of trisomy and congenital anomalies does not prove a causal relationship. It is of interest that trisomy of No. 22 was found in an individual with Sturge-Weber syndrome.[20]

DR. HAMBURGER: Up to now we have been discussing instances in which extra chromosomes are present. Of equal interest are the chromosomal deficiencies. Gonadal dysgenesis or Turner's syndrome, although originally predicated on the basis of the triad of short stature, multiple congenital anomalies and gonadal dysgenesis, has achieved more solid footing since an XO karyotype was observed in a high proportion of cases. Dr. Guevin, what is the explanation for this XO chromosomal complement?

DR. GUEVIN: The XO chromosomal complement in Turner's syndrome results from non-disjunction during spermatogenesis with subsequent fertilization of an X ovum by a sperm without any sex chromosome.

DR. HAMBURGER: For a brief interval in the development of our understanding of chromosomal disorders, it seemed that the XO karyotype might be the most appropriate ground on which to define the limits of Turner's syndrome. The observation that chromatin-negative patients with Turner's syndrome had an XO karyotype was compatible with the prevailing concepts of sex differentiation that two X chromosomes are necessary for ovarian development, and that agonadal individuals develop in an immature female fashion. However, frequent reports of so-called varients of Turner's syndrome are a source of considerable confusion. Dr. Guevin, would you please describe some of these perplexing patients?

DR. GUEVIN: Twenty to 30 per cent of patients with typical features of

Turner's syndrome are chromatin-positive, and they presumably have an XX karyotype.[3] Also chromatin-negative individuals with an XO karyotype have menstruated regularly[31] and, in a carefully documented study, one such subject has given birth to a normal chromatin-negative male.[32] Males with undescended or dysgenetic testicles and the anomalies and stature of Turner's syndrome have been reported with an XY karyotype,[4,46] and in one instance with an XO karyotype.[34] More recently Grumbach[10] has reported a patient with Turner's syndrome and an XO chromosomal complement, but the buccal smear was chromatin-positive.

DR. HAMBURGER: Upon what basis might these observations be integrated into a unifying concept for Turner's syndrome?

DR. GUEVIN: The development of a unifying concept to encompass these seemingly incongruous observations, and perhaps others still to be described, necessitates thinking in terms of genes rather than of buccal smears or chromosomes. The fundamental factors controlling gonadal and somatic development clearly reside within genes. With this principle in mind, and recalling the various mechanisms by which mutations may be produced, possible explanations for the Turner's varients may be considered.

The chromatin-positive XX individuals at times have one X which is smaller than its mate.[35] Perhaps this represents the deletion of genes from the smaller X, leading to gonadal and somatic anomalies and a retention of the heterochromatin and chromatin positivity. Or both X chromosomes with the full number of genes could have functional impairment of the latter, perhaps on a biochemical or enzymatic basis. Some chromatin-positive patients with Turner's syndrome are XX/XO mosaics.[22] The menstruating and fertile XO subjects might be examples of translocation of genes from the missing X to an autosome, thus retaining relatively normal genetic capacity for gonadal and sexual development. The loss of the remaining genes from the missing X could be the basis for the somatic anomalies. Another possible explanation in these XO patients is an erroneous karyotype. Since the X chromosome cannot be separated with absolute certainty from autosomes of pairs 6 through 12, both X's might be present and one autosome absent, the latter resulting in somatic anomalies. The chromatin-positive, XO patients might have retained the heterochromatic portion of the missing X chromosome by means of translocation to an autosome. Similar explanations could be applied to each of the variants of Turner's syndrome, employing combinations of known mechanisms to account for the observed karyotype and phenotype.

Hence, it becomes apparent that the possession of an incomplete complement of functioning genes on the sex chromosomes is the fundamental factor common to all of these disorders. The involved genes might be those for sexual or somatic differentiation, or both. The karyotype reflects the qualitative and quantitative aspects of this deficiency, and the phenotype is the expression of these differences. Thus, Turner's syndrome should be considered a spectrum of disorders (Table 1) at one end of which are females with classical Turner's stigmata, an XO karyotype, but remaining fertile. At the other extreme are males with similar anomalies, an XY karyotype and, possibly, fertility. Between these extremes would fall the remaining variants.

Table 1

Classification	Sex Chromatin	Karyotype	Phenotype	Anomalies
Turner's	—	XO	Fertile	Yes
Turner's	—	XO	Immature Menstruating	Yes
Turner's (Classical)	—	XO	Immature	Yes
Turner's	+	XX,Xx XX,XO XO	Immature	Yes
Pure Gonadal Dysgenesis	—	XY	Immature	No
Turner's—Male	—	XO	Cryptorchid Hypospadiac	Yes
Turner's—Male	—	XY	Cryptorchid Dysgenetic	Yes

DR. HAMBURGER: What is the karyotype of the true hermaphrodite? Dr. Fruchtman, can you help us?

DR. FRUCHTMAN: True hermaphrodites of course, have both ovarian and testicular tissue. Several karyotypes have been reported, including XX, XX/XO, XXX/XO and XXY/XO.[37,40] However, most reported cases have had 46 chromosomes and XX sex chromosomal complement.

DR. HAMBURGER: What is the genetic significance of the XX karyotype in the true hermaphrodite?

DR. FRUCHTMAN: It is of interest that hermaphrodites lacking the Y chromosome have been able to develop testes. This is in contrast to Harnden's case of pure gonadal dysgenesis in which the karyotype was XY and no gonadal tissue of any type was found.[33] Such cases challenge current concepts of sex differentiation which propose that testes do not develop without the Y chromosome and, as a corollary, testes do develop when the Y chromosome is present. Again, one must think in terms of gene dysfunction, translocation, deletion or combinations of these or perhaps other mechanism to explain such occurrences.

DR. HAMBURGER: Dr. Guevin, are there instances of autosomal deletion?

DR. GUEVIN: Turpin[22] has reported a 4 year old boy with multiple vertebral anomalies and 45 chromosomes. Missing was one of the group of small subterminal chromosomes. In addition, one of the larger subterminal chromosomes was remarkable in that it had much longer "arms" than its homologue. Perhaps this was the result of partial translocation with the deletion of remaining fragments.

DR. HAMBURGER: What observations have been made on the chromosomal complements in leukemia? Dr. Guevin, would you briefly answer this last question?

DR. GUEVIN: The increased incidence of leukemia in mongolism has resulted in considerable interest in the study of the chromosomal complements. In the

chronic leukemias they have been normal, but in acute leukemia abnormal chromosomal counts have been reported in two patients, and evidence for translocation existed in two others.[41,42] An additional patient with presumed radiation-induced acute leukemia had a modal number of 42 with many polyploid cells containing predominantly multiples of 21 chromosomes.[41]

DR. HAMBURGER: Dr. Smith, what would appear to be the future for studies of the chromosomes?

DR. SMITH: Your associates, Dr. Hamburger, have been quite effective in answering questions, and I judge that the three of you are better qualified to discuss this aspect. One must have solid footing in the knowledge of the past and present before attempting any projection into the future.

We have referred today to the gene as the final locus of intrachromosomal determinants for cellular development and function. It is tempting, therefore, to propose that inquiry into the nature of this genetic unit would be potentially fruitful. There is little question but that the assumption of the existence of such a genetic unit has served well to keep the peace between morphology and function. However, the evidence is fast accumulating, if not already at hand, that genes in the traditional sense do not exist. The brilliant contributions of the past few years in respect to the chemistry and molecular configuration of deoxyribonucleic acid (DNA), the substance which carries the master code of genetic information, permit us now to visualize for the first time the elemental structure of the system for transmission of cellular information. Instead of the mysterious gene, we now have a well-defined chemical template, a long spiral ladder of but three chemical moieties — sugar, phosphate and purine-pyrimidines.

The enthusiast might say that much of the future effort will be concerned with correlating the orderly structural variations of DNA with specific chromosomes. He might ask: what are the forces which control the pre-mitotic segregation of DNA material into the exact numbers of chromosomes characteristic of each species? However, these are tremendous challenges for ultra-micro physical chemistry, and the answers would seem to lie at the far end of such a long projection into the future.

As clinicians, we are faced with a great number of disorders, many poorly understood as to etiology, with such general designations as inherited, acquired, congenital, idiopathic, familial, constitutional, inborn error of metabolism, etc. The opportunity is unlimited to attempt a genetic deciphering of these in terms of a demonstrable defect in one or another of the autosomes. This, of course, means a painstaking effort at correlating specific autosomes with specific cellular and organ systems. It would seem to be a large area for future research. Progress in unravelling some of the mysteries surrounding sex differentiation has been possible by virtue of the geneticist's ability to recognize the sex chromosomes. No ready tag of identification is apparent for the autosomes but, judged by the resourcefulness shown to date, we can be confident that new contributions of significance will be added to this rapidly advancing field.

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MECHANICS OF AN UNUSUAL BASILAR FRACTURE

WILLIAM E. LOECHEL*

Several years ago, while affiliated with the National Institutes of Health in Bethesda, Maryland, the author was engaged in the production of a head model comprising several transverse sections. In an effort to delineate accurate relationships, a cadaver head was obtained from the University of Maryland, Department of Anatomy.** Plans called for sectioning this embalmed head transversely on one side only, and to retain the unsectioned half for future use in another project.

A mid-sagittal cut was made of the embalmed (and frozen) specimen and the right half was sectioned transversely. Transparent sheets of acetate were placed upon the upper surfaces of each slide and the contours of the structures drawn on the acetate with a fine pen. It became apparent after a short while that identifying the cut muscles was a confusing experience when it should not have been. Bony landmarks were all well defined and many muscle masses were in appropriate locations. However, the presence of large expanses of unidentifiable substance (appearing to be muscle) prompted closer inspection to the boundaries of the known structures, especially the known muscle sections. So bizarre were the relationships that the uncut half of the head was examined to see what these unidentified areas looked like on the mid-sagittal plane.

An examination of this surface (Fig. 1) showed a 4 centimeter space between the basilar process and the atlas. The cervical vertebrae were in proper alignment and the altanto-occipital ligaments were intact beneath the body of the atlas and a portion of the anterior lip of the foramen magnum. The "unidentifiable" areas were obviously extravasated blood.

It was suspected that the condyles and other lateral structures would also be found displaced downward; this was confirmed by an x-ray and laminography (Fig. 2).

Other significant relationships seen in the sagittal section were (Fig. 3)

1. Cord transected and constricted below the section, as though the victim's head had dropped forward after the initial violent hyperextension.
2. The hypophysis was severed at its stalk; the tentorium was still intact.
3. The optic chiasma was displaced backward.
4. The superior margin of the corpus callosum presented extremely unusual contours along the posterior one-half.

Condyles, hypoglossal canals, transverse processes and a lip of clivis were all broken loose as a unit from the base of the skull; (Fig. 4) the atlanto-occipital and

*Director, Medical Illustration Department.

**Procured according to Maryland State Anatomy Law from University of Maryland, Department of Anatomy for use by the author while at the National Institutes of Health, for project of National Institute of Neurological Diseases.

atlanto-epistropheal ligaments (as well as the other cervical structures) were properly related.

In view of these findings, it is interesting to ponder the various degrees of intra-cranial damage that could be associated with cervical fractures and/or dislocations.

Figure 1

Basilar process and atlas. (a) Transection of cord.
(b) Severed hypophysis. (c) Displaced optic chiasm.

Figure 2

Downward displacement of condyles.

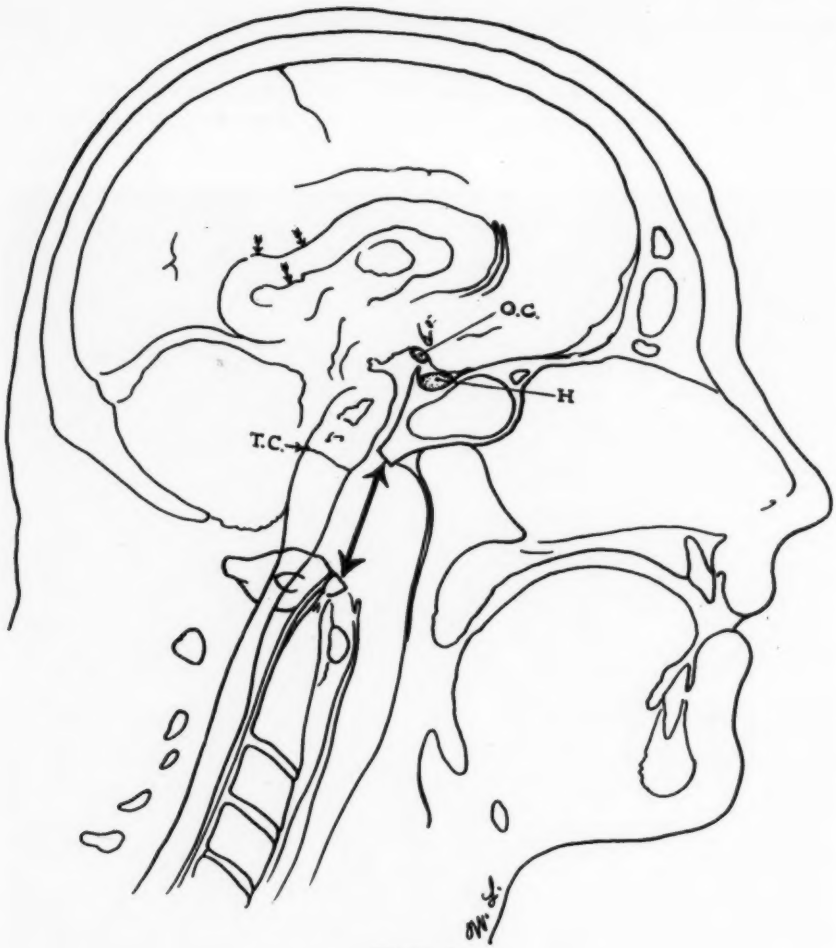


Figure 3

Tracing on glass of mid-sagittal surface. The heavy arrow indicates interval of displacement. (H) hypophysis. (o.c.) Optic chiasm. (T.C.) Transsected cord.

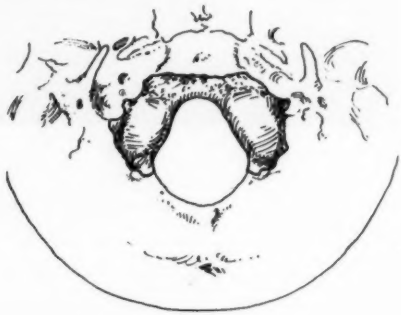


Figure 4

Condyles, hypoglossal canals, transverse processes and lip of clivis broken loose.

MEDICAL ILLUSTRATIONS: HINTS FOR THE PHYSICIAN-AUTHOR

WILLIAM E. LOECHEL*

Well planned manuscripts, lectures, and exhibits effect economy and prestige. It has been stated that 90% of what man learns he learns through his eyes; which, in the case of illustrations for scientific work, means clarity and accuracy as opposed to suggestion or impression.

The initial steps in utilizing visual aids in scientific writing are the *productive* steps which, if planned properly, make *reproductive* phases relatively trouble free. The object of this article is to present suggestions to the physician intending to use pictures with his text or exhibit — suggestions that will permit his fullest control as the initiator and will make for minimum delay in processing his ideas.

ILLUSTRATIONS

Although the medical illustrator is happy to discuss proposed illustrations on an informal basis, the necessary formality of a written request has every advantage. Such a request will establish priority and eliminate confusion relative to the date on which the finished work is due. The general scope of the work should be indicated, and notation made as to the purpose of the drawing. This is important because there are instances when one drawing can be prepared to serve several purposes. On the reverse side of the service request card, the time spent on that particular project may serve as useful information in a future undertaking of a similar nature (Fig. 1).

When illustrations are to be made during an operative procedure, it is obvious that a good vantage point for the illustrator is to everyone's benefit. This does not mean that he need be beside the patient, for there are other equally practical places to have him stationed, such as behind the anesthesia screen or on a high stool behind the operator or assistant. It should be pointed out that the illustrator who is the sole tenant of the space atop a stool is more apt to get better pictures with fewer questions asked than the illustrator who first at the table, then elsewhere, and intermittently back at the table.

Considering the audience who will view a picture and the time spent by the viewers will, to some degree, determine the nature of the illustration. It is a waste of facility to produce a complicated drawing when comprehensive simplicity serves the purpose better. As an example, the person listening to a speaker (and seeing slides) would have an extremely brief interval to digest new information or grasp a great amount of information that covered the entire screen. In this instance a simple, bold picture is effective, stimulating, and instantly understood (Fig. 2). The reverse is true in the case of an exhibit illustration where the viewers are prepared to spend as much time as they feel necessary. In the case of books and journal articles, the choice depends on space.

*Director, Medical Illustration Department.

TIME RECORD

DATE	ILLUSTRATOR	HOURS

Figure 1

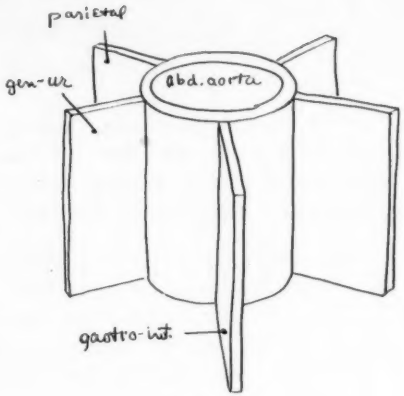


Figure 2

While the average physician will not attempt to prepare his own rough drawings, he may, nevertheless, take advantage of tracing available reference material in order to provide the illustrator with a definitive idea. This cannot (and need not) always be done, but there are many times when ordinary typewriting paper can be placed on a book illustration and a reasonable series of contour lines drawn. It should be remembered that this is a "rough" only, but it will provide good landmarks for the additional information to be incorporated in it (Fig. 3).

Quite often specimens from the autopsy room are found unusual enough to warrant drawing. If it is at all possible, the doctors involved should call the illustrator to stand by as the specimen is being cut. If need be, several drawings can be made during the entire sectioning or inspection and the illustrator will have less difficulty in reconstructing the tissues. There is the obvious advantage of having the illustrator draw from the fresh specimen or briefly fixed specimen as opposed to the long stored and mis-shapen one.

If the occasion arises when a surgical procedure must be done late at night or on a week-end, and if this procedure yields noteworthy information that lends itself to drawings, the surgeon should do one of two things; i.e., call the illustrator or dictate a detailed description as soon as time permits. The problem of creating a lifelike field from the written word is not as difficult as it might sound.

Reference pictures that may be provided the illustrator should have the stamp of authenticity; it is easier to simplify than to build up. As an example, the face of the man during an epileptic seizure was modified from a photograph and presented no problem at all; whereas great difficulty would have been encountered had a "doodling" been the foundation for the finished drawing (Fig. 4).

At appropriate times, non-medical illustrations can be a delightfful way to emphasize a point. This is especially true when presenting information to non-specialized audiences (Fig. 5).

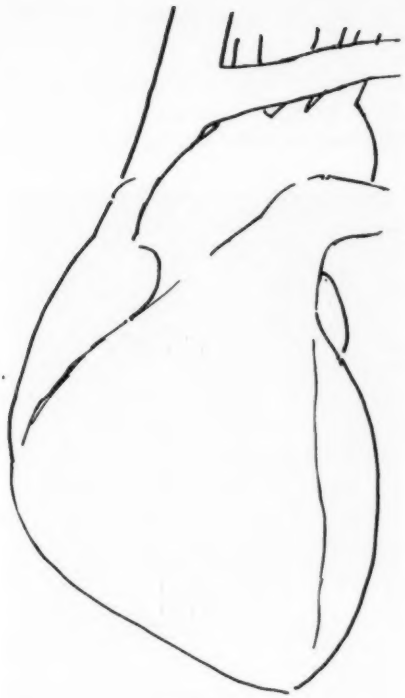


Figure 3

Figure 4

Reprinted, with permission, from the American Journal of Nursing, March 1957.

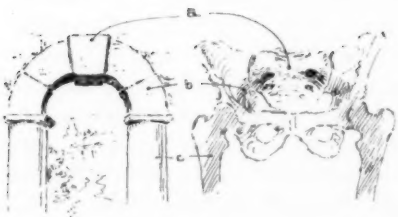


Figure 5

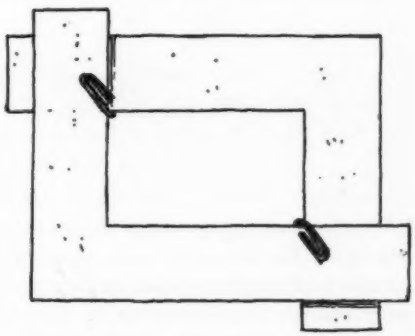


Figure 6

When the physician has gotten together a complete article and all the illustrations, he may wish to "crop" them himself. Not infrequently he will possess more photos than drawings and these are probably routinely sent to the publisher to be printed as is. Much space may be wasted; many times the focal point is minimized. If a simple frame (angles) and paper clips is devised, this will provide an adjustable window for him to view each picture as he would like it to appear in print. It

is surprising how much can be omitted, all to the good of emphasizing the main point of interest (Fig. 6).

Photography and drawing can combine beautifully. This is an especially useful technique to use where a series of subjects is to be shown. It is far less trouble to print six photos and do art work on them than to make six slightly different drawings of the same subject (Fig. 7).

Figure 7

There are some typewriters which produce light open letters which very closely resemble neatly done hand-lettering. If such an instrument is available, the physician can have the labels typed and arrange them as he thinks best by rubber cementing them to the drawing. These can be removed and/or replaced without having to alter the drawing per se (Fig. 8).

When sending drawings or photographs to the publisher, it is recommended that these be backed by heavy cardboard and not affixed to the appropriate page in the manuscript on manuscript paper. It is, of course, a risk to write on the reverse side of a photograph as the impression has been known to appear as an imperfection in the printed picture (Fig. 9).

In planning illustrations for a book, it makes for easier reading if illustrations of comparable technique are near each other. This need not mean that half-tone drawings and photos cannot appear side by side with line drawings, but it does imply that delicacy juxtaposed to boldness is undesirable.

A friend or patient may be an excellent commercial artist for a newspaper, and he should be allowed to remain an excellent commercial artist for a newspaper.

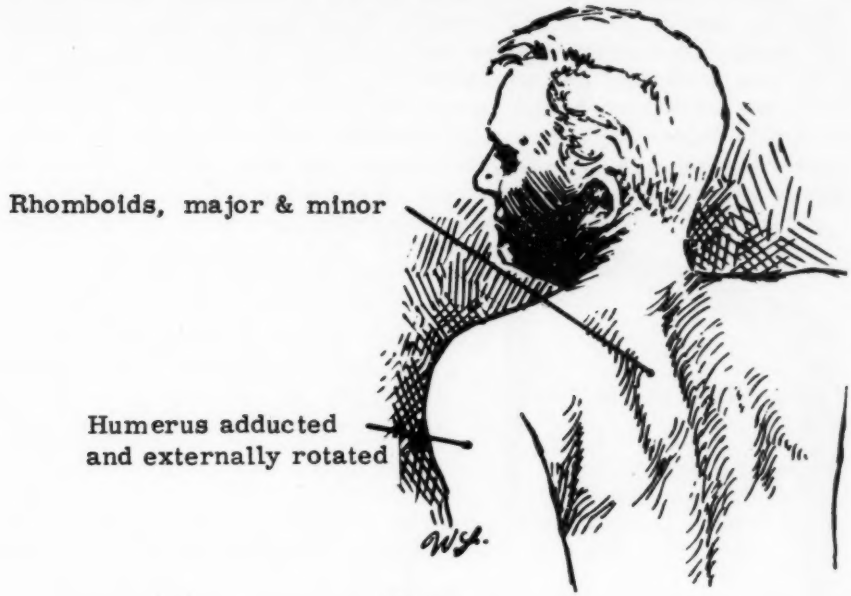


Figure 8

Reprinted, with permission, from Kendall, H. O., and Kendall, F. P.: Muscles, Testing and Function, Baltimore, Williams and Wilkins, 1949.

This comment is not meant to be acrimonious, and there are obviously acceptable variations to the rule. But, by and large, a specialist will have little trouble in executing an assignment quickly.

One drawing may impart information that could not be presented effectively by any other means. As an example, several photographs of a dissected brain showing structures associated with the visual system would be far inferior to a semi-diagramatic drawing utilizing information from clinical studies, etc. (Fig. 10). In this particular instance it was necessary to develop a system of dot and line patterns to carry the retinal information back through the structures depicted, but this same drawing could be reproduced as a negative slide and color added for more ready visualization. If the drawing requires labels, it is far more satisfactory to indicate them (precisely) on an overlay sheet so that the illustrator will have no problem in directing the leader line or arrow to the exact spot. All too often, especially in photomicrographs, a grease pencil is used on the acetate cover to indicate where an arrow should point. These grease pencil markings are not accurate enough in most cases; it is better to use a straight pen and India ink. In all instances it is better to be hyper-critical or painstakingly exact in preliminary work and the physician who constructively criticizes sketches prepared for him is a much appreciated patron. It is too late, when the article is in print, to apologize for a quickly done piece of art work or a carelessly planned picture. The instances when such drawings are redone for future publicition are rare.

Doctors planning to have illustrations prepared should decide basically whether they need color or line or tone drawings. Where topography only is important, it is foolish to have a colored picture made. Color work takes longer and will often times lack the depth of field when printed as a black and white picture. Furthermore, line drawings can be reduced with relative freedom and can be printed on a variety of paper stock. Half-tones are reasonably adaptable to variation in size and quality of paper stock, but color work requires very special handling and can be printed satisfactorily only on coated stock.

Figure 9

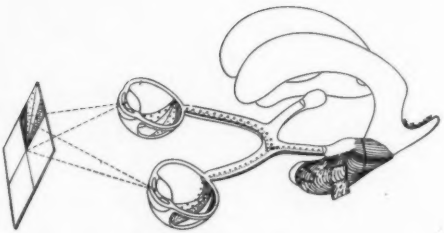
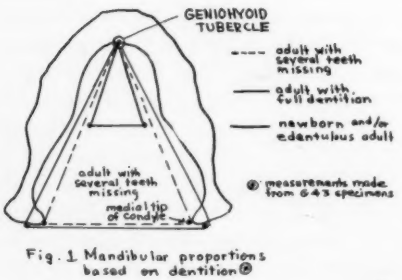


Figure 10

Reprinted, with permission, from Brain 81:38, 1958.


GENIOHYOID TUBERCLE
adult with several teeth missing
adult with full dentition
newborn and/or edentulous adult
measurements made from 643 specimens
adult with several teeth missing
medial tip of condyle
Fig. 1 Mandibular proportions based on dentition

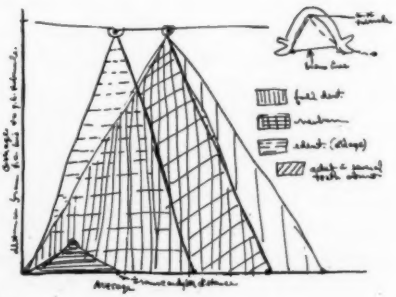


Figure 11

CHARTS AND GRAPHS

Again, a written request should stress pertinent data and the intended use. Some journals have specific requirements as to size, etc., and it is well to know this before drawings are begun. Ideal for dvelopment of a graph is blue line or green line graph paper. Above all, avoid using rough paper. Attempt to relieve plotted information with a more meaningful presentation if possible. Here we show one set of facts in two ways. The second obviously has more impact — and incidentally makes use of the book tracing mentioned previously (Fig. 11).

An ordinary pencil of medium softness permits easy erasures. Colors can be indicated, or colored crayons can be used when "rough" is completed.

You might wish to select a plastic template that suits your needs. There are numerous kinds available with such things as arrows, benzene rings, flasks, etc., or a sheet of clear x-ray film can be used, appropriate and much used symbols being cut with a razor blade or Bard-Parker knife

Commercially available symbols can save lots of time if you do your own inking. A variety of numbers, arrows, symbols can be purchased which, being wax-backed, can be pressed on the picture or chart. Special sheets can be made up. It is a good idea to get the publisher's views by reading his guide-book.

Use commercially available gummed tapes for large wall charts that you want to do yourself. All colors and patterns are available in many widths, and a simple series of bars or lines can be applied to cardboard or blackboard in less time it would take to show the illustrator what is wanted

Write "top", size, and typewrite legends when you send your illustrations to the publisher. There are occasions when a record of some kind reaches him and of its very nature there are no numbers or letters to tell him how or where to place a picture. If you want to borrow from a publisher, it is better to borrow the engraving than to re-photograph the printed reproduction. Obviously, the clearest print will come from the original plate.

EXHIBITS

The author is prime mover and final judge. The illustrator is his agent for getting other wheels of motion started. (If either becomes disengaged, there is apt to be a mess instead of a mesh).

Especially important are the names of all persons contributing information for its production. Provide correct duplicate typed copy to all co-authors and to the Illustration Department.

Hours spent on a scale model may save weeks on the full scale product. Label difficult to identify material carefully and consistently. This is especially true of x-rays, which should be marked "top" and should have the location (according to the scale model exhibit) written on tape that is affixed to them.

If you take your own pictures, try to provide a neutral or contrasting background.

Humility can be over-done; bigness can belittle you. Many times excellent material is displayed in an exhibit hall in a manner that suggests haste, poverty, or lack of enthusiasm. At other times, one sees the reverse. A poor design can ruin an excellent message; a good design can enhance an excellent message.

Plan weekly conferences and inspection sessions. Again, it is to be stressed that an hour spent may be 10 hours saved.

Whether it be a drawing, chart, photo or exhibit, the physician-author will find it an exhilarating experience if he will utilize ways to prepare work for the illustrator.

EMERGENCY MANAGEMENT OF THORACO-ABDOMINAL TRAUMA; A STUDY OF 275 INJURY PATIENTS

JOSEPH L. PONKA, M.D.* AND ROBERT O. ANTONI, M.D.*

The purpose of this paper is to consider the patient with thoracoabdominal trauma, and to analyze the injuries of 275 patients occurring in a four year period, 1953-1956. Crushing injuries to the chest and upper abdomen, chest trauma, penetrating wounds and severe upper abdominal injuries will be discussed. It can be seen that for the moment attention will be directed to the thoracic and abdominal viscera. It will be recalled that, in injuries to the chest, abdominal organs may be injured, and vice versa; in upper abdominal injuries the thoracic contents or diaphragm may be damaged.

In order to emphasize the fact that certain injuries result in injury to multiple organs, we have utilized simple drawings. The path of a bullet is illustrated in Fig. 1, the effect of multiple stab wounds is shown in Fig. 2, and the result of a heavy crushing force is shown in Fig. 3.

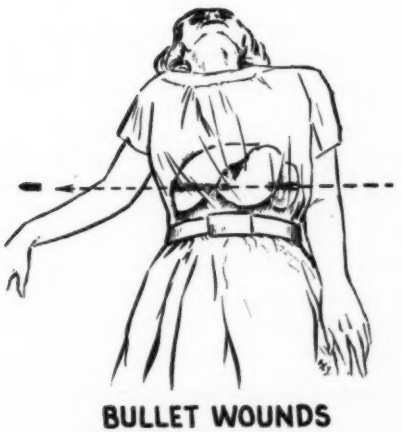


Figure 1

Bullet wounds should at once lead to the suspicion that multiple organs have been injured. Prompt surgical intervention is necessary for control of hemorrhage and repair of injured viscera.

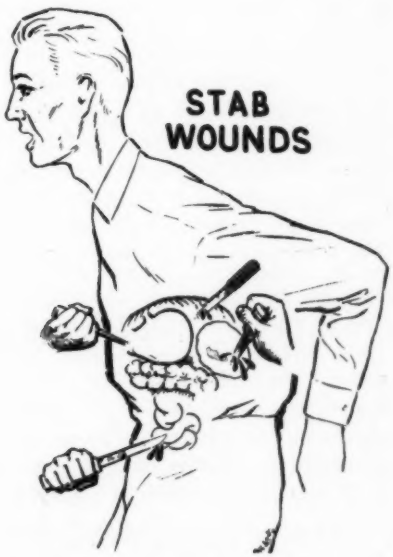


Figure 2

Stab wounds result in penetrating wounds to multiple organs. It is often impossible to determine the direction and depth of penetration clinically. Early surgery is mandatory. Meticulous exploration of the peritoneal cavity and its contents is essential.

*Division of General Surgery.

Figure 3

Heavy objects crushing the patient against a wall, or falling on the patient, or automobile accidents, are likely to result in multiple organ injuries, as well as rib fractures.

Obviously, the first concern is the preservation of the life of the patient. Extensive and severe multiple injuries require the presence and intensive activity of two or more doctors until the acute problem is under control. Since the patient can survive but a few minutes without oxygen, an airway must be provided at once. This may require only supporting the lower jaw in an unconscious patient, or aspiration of secretions in the nasopharynx, or it may call for tracheostomy, depending upon the needs of the patient. Closure of "sucking wounds" of the chest must be immediate, thus avoiding a fatal pneumothorax.

The maintenance of circulation is also of the greatest importance. Bleeding must be stopped at once. This can be done, in some instances, with direct pressure applied over a sterile gauze pad. Strangely, this simple expedient is so often overlooked. Pressure dressings will control bleeding from oozing areas; tourniquets may be used for periods of approximately two hours if major arteries in the extremities are not readily accessible for clamping. Continuing loss of blood into the thorax or abdomen must be stopped even if the condition of the patient is poor, for it will never improve if exsanguination continues.

While the airway is being provided, and bleeding is being controlled, intravenous fluids are given, and arrangements for transfusing the patient are proceeding hastily. Plasma, or plasma expanders, are given to those patients whose blood volume is dangerously low, while whole blood is being secured. Hemorrhage demands replacement of the red cell mass.

During the above period the patient's pulse, blood pressure, respirations and temperature should be recorded, and retaken at short intervals as indicated by the needs of the particular patient. There is no substitute for such objective evaluation of the vital function of a seriously injured patient.

Once the patient is breathing, and has a satisfactory blood pressure, we begin a careful appraisal of the patient's injuries. A careful history is helpful.

Falls from great heights, severe automobile accidents, and crushing injuries usually result in multiple-organ injuries. Fig. 3. These are most serious. Accompanying head injuries are of serious prognostic significance. Remember that patients injured in falls may be diabetics, alcoholics, hypertensives, epileptics, or psychotics. At times the patient cannot be of help; he may be unconscious, or seriously injured and unable to give an account of what occurred. Hence, any information gained from informed sources is helpful and appreciated. The entire patient is carefully scrutinized, but of course the injured parts are subject to special attention. The patient must be disrobed completely for proper examination. This obvious detail is too often overlooked. X-ray studies must not be ordered too quickly, for they are not life-saving.

Morphine continues to be most useful in controlling pain and lessening apprehension. Neverthless, respiratory depression is an undesirable side-effect which can be serious if the dosage is too large or repeated at short intervals. Morphine should not be given if its administration would add confusion to the attempted differential diagnosis. Anoxia may result in extreme restlessness, even mania. Morphine, in this instance, may further depress respiration and end in disaster.

Thoracic injuries of interest here are multiple rib fractures, (Fig. 4) injury to the lung, hemothorax, pneumothorax, hemopneumothorax, and associated injuries of the liver, spleen, or kidney, stomach or pancreas or heart.

Severe chest trauma with bilatetral rib fractures produces paradoxical movement of the thoracic wall. This causes serious disturbances in intrathoracic pressure, and hence in the pulmonary exchange of air, as well as in filling of the heart.

These disturbed functions vary directly with the severity or degree of the paradoxical motion. Pneumothorax, hemothorax, or bronchial obstruction may be additional complicating factors present. In the management of such injuries we must do the following promptly:

1. Provide an unobstructed airway.
2. Aid in expansion of the lung.
3. Provide an intact thoracic wall if there is an open pneumothorax.
4. Stabilize the chest wall against paradoxical respiration.
5. Combat hypotension.

Penetrating wounds may cause hemorrhage and pneumothorax. Sucking wounds should be treated at once with an occlusive dressing or surgical closure. Hemothorax may be remedied by thoracentesis or thoracotomy and placement of an underwater thoracotomy tube. Small amounts of hydrothorax, pneumothorax, or hemothorax, not embarrassing respiration, may be observed carefully.

Tension pneumothorax develops whenever air enters the pleural space on in-

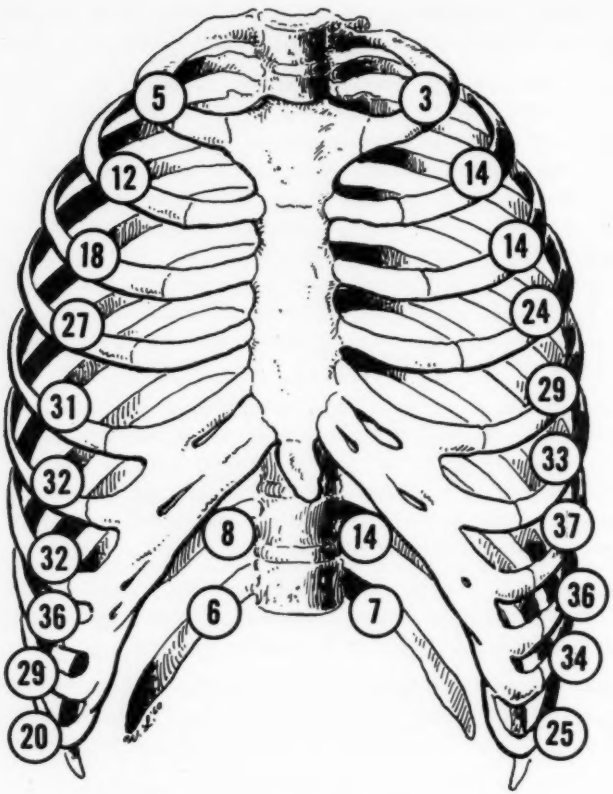


Figure 4

Rib fractures are often multiple, especially in falls, automobile, or crushing injuries. Greater direct force is necessary to fracture the upper ribs because they are better protected. The lowermost ribs are relatively mobile, and are fractured only when subjected to severe direct trauma. When the lower ribs are fractured, injury to the spleen, liver and kidneys, must be suspected. In our experience with 275 injured patients ribs 4-10 inclusive are fractured most frequently, as indicated in this illustration.

spiration, only to be retained on expiration. The intra-pleural pressure increases, the mediastinum is displaced to the opposite side, further interfering with pulmonary function. This also has an adverse effect on cardiac function. Here, either aspiration of the air, or thoracotomy tube drainage are corrective. Pain should be controlled by intercostal block, or opiates. Strapping tends to restrict motion of the thoracic cage, and we do not generally use this method for control of pain. Codeine is a good analgesic for simple rib fractures.

It should be remembered that subcutaneous emphysema is an indication of one of the following:

1. Puncture of the lung, pleura.

2. Ruptured trachea.

3. Ruptured esophagus.

We feel that such patients should have relief of the pneumothorax which usually accompanies the above injuries, but which may be difficult to demonstrate because the subcutaneous air presents a confusing picture on x-ray examination.

Cardiac tamponade, caused by bleeding into the pericardial sac, is another emergency situation. Hypotension, distended neck veins, rapid thready pulse, suppressed heart sounds, and an enlarged heart shadow are the important findings in this condition. Pericardial tap should be done at once, and if aspiration does not correct the situation, then surgery becomes necessary to stop the bleeding. Here ill-advised over-transfusion may result in fatalities when such patients are managed by the uninitiated.

We wish to emphasize that wounds involving the chest, whether penetrating or not, may damage the liver, kidneys, spleen, pancreas, or stomach. Conversely, wounds involving the upper abdomen may result in laceration of the diaphragm or lung. Upon subsequent abdominal section an open pneumothorax is produced. The implications as to need of endotracheal anesthesia are obvious. In wounds that have penetrated the lower chest or upper abdomen, persistent hypotension, abdominal tenderness or rigidity, absent bowel sounds, peritoneal fluid, or pneumoperitoneum, indicate the need for laparotomy. Penetrating upper abdominal wounds demand surgical intervention as soon as the patient's condition will permit. Lacerated viscera must be repaired promptly. Anesthesia is generally best administered by way of an endotracheal tube in this group of patients.

More treacherous are the closed abdominal wounds. Here the surgeon must possess a high index of suspicion, and he must follow these patients most carefully if the usual high morbidity and mortality rates are to be avoided. Marked tenderness, rigidity, pallor, rapid pulse, hypotension, absent bowel sounds, elevated white cell count are most significant findings. Abdominal x-ray may show pneumoperitoneum, or peritoneal fluid.

With the above findings surgery is carried out as soon as the condition of the patient is satisfactory. In doubtful cases we advise surgery, for it is well known that such patients die of peritonitis if neglected. We salvaged two patients in one year having closed abdominal wounds. One had a perforated duodenum, the other a perforated ileum.

It is well known that following abdominal injury the fixed viscera, (liver, kidneys, spleen) are more commonly injured. Lacerations of the intestinal tract are likely to occur at the points of attachment to the posterior abdominal wall, as, for instance, the jejunum near the ligament of Trietz, the ileum near the cecum, and the sigmoid. A distended viscus, such as a full bladder or intestine, is in greater danger of injury than if it were collapsed. Pre-existing disease also increases the danger of rupture.

At surgery the opening in the viscus is closed after the contents of the peritoneal cavity have been aspirated. Great care should be taken to remove all particulate matter.

Small openings in the large bowel may be closed with drainage. Larger lacerations of the colon may require exteriorization of the colon or colostomy proximal to the laceration. Early surgery is the key to success. The surgical exploration must be painstakingly thorough.

At times, injury to the spleen may produce a confusing picture. Whenever the spleen is lacerated and bleeding continues, the need for surgery becomes apparent. The usual clinical signs and symptoms include abdominal and left shoulder pain, palpable mass in the left upper abdomen, and abdominal distension. Of course, we look for the rapid pulse and falling blood pressure. Roentgen examination may reveal increased density in the left upper abdominal quadrant, obliteration of the splenic shadow, elevation of the left diaphragm, displacement of the stomach toward the right, or evidence of fluid in the peritoneal cavity. Greater discrimination is necessary in order to avoid missing those cases of delayed rupture of the spleen, i.e., those cases occurring forty-eight or more hours after injury. Such patients must be observed at close intervals. Treatment is splenectomy.

It is apparent that severe lacerations of the liver need more exacting treatment than mere packing. This method may act as a wedge and the remaining portion of the liver is further disrupted. (Fig. 5.) The better approach to severe liver lacerations includes:

1. Better exposure of the liver, using a thoraco-abdominal approach where needed. We believe that this approach should be used more frequently.
2. Suture ligature of individual arteries, veins and bile ducts with fine silk sutures.
3. Adequate approximation of the liver surfaces after using oxycel or gelfoam judiciously.
4. Multiple drains are important.

It is important to remove hopelessly devitalized liver fragments. Remember that the liver has a great capacity for recovering following trauma.

The kidney should also be considered in this group of injuries. Hematuria is an important finding. Intravenous pyelography is done in these cases first. If there is poor visualization by this technique, then retrograde pyelography is carried out. Hematuria alone is treated conservatively, whereas hemorrhage and severe damage to the kidney require surgical intervention. Simple drainage and oxycel packing are used in suitable cases. Falling blood pressure, rapid pulse and falling hemoglobin or hematocrit values suggest need for surgery. Nephrectomy is done if the kidney cannot be salvaged.

In any of the above patients paralytic ileus may be an annoying complication. The use of a Levine tube is most helpful. Restriction of oral intake, heat applied to the abdomen, enemata, and use of the rectal tube, will generally correct the ileus. In some instances a Miller-Abbott tube must be employed. Delay in insertion of this tube is not an uncommon error. Hydration is accomplished by use of intravenous fluids.

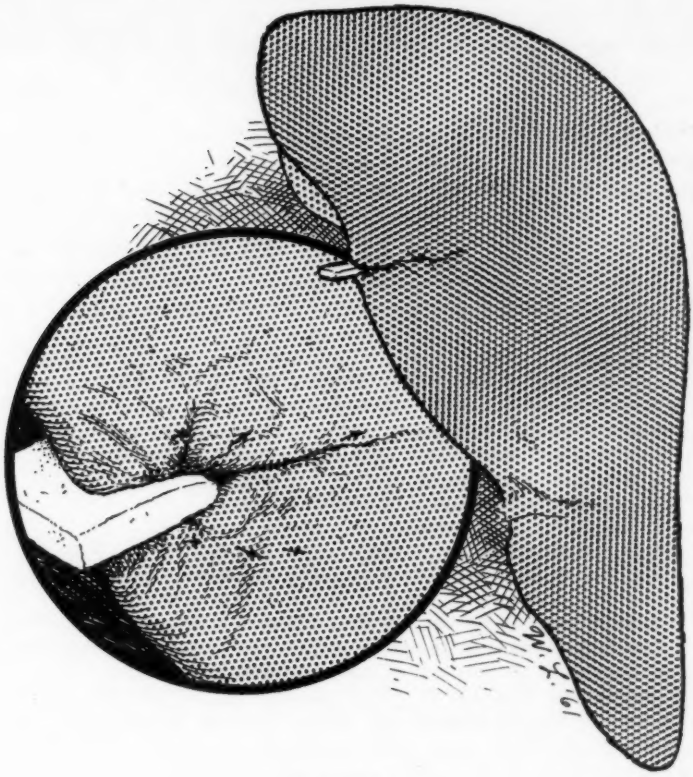


Figure 5

Crushing wounds over the liver, or falls from great heights, may result in stellate lacerations of the liver. This drawing emphasizes that "packing" the liver with hemostatic materials may act as a wedge, further disrupting the friable liver. Forceful packing of liver lacerations with packing of any type is not recommended.

Finally, we wish to emphasize that in severely injured patients the knowledge and assistance of several doctors is necessary. This is obvious, since several diagnostic and therapeutic procedures must be going on at the same time. Consultation with the various specialties is readily available and valuable and should be utilized whenever needed.

PROGNOSIS: It is apparent from TABLE 1 that the prognosis in thoraco-abdominal injuries becomes more grave as the number of organs injured increases.

In severe crushing injuries, automobile accidents, bullet wounds, and falls from great heights, the prognosis is grave because multiple organs are injured. Eleven patients expired. Five were unable to survive because of extensive blood loss secondary to injuries of the liver, spleen, kidney and multiple fractures. Four patients had severe head injuries and died as a result of cerebral damage. One patient died of peritonitis, another of pulmonary embolus.

Table 1

INCIDENCE OF ASSOCIATED VISCERAL INJURIES IN 275 PATIENTS (1953-1956)

	No. of Patients	Expired
Spleen	18	2*
Kidney	6	2*
Diaphragm	6	2*
Intestine	5	0
Liver	5	3*
Stomach	4	2*
Multiple Injuries	50	11

*Patients with Multiple Injuries.

SUMMARY

In the emergency management of major trauma there are several very important and consistent features. In order that these may not be lost in a lengthy treatise, we would prefer to summarize them as follows:

1) In major trauma the *preservation of life* is our first consideration. An *adequate airway* and *maintenance of circulation* are our first objectives in supporting life.

2) X-ray examination should not be generally carried out until the patient is out of danger and his respiratory and circulatory functions have been stabilized.

3) In injuries of the chest we should provide an unobstructed airway, expand the lung, make the thoracic wall intact, drain the pleural cavity, relieve pain by intercostal block, and combat infection.

4) In injuries of the *lower chest* or upper abdomen we must not forget injuries to *adjacent organs* or viscera.

5) The spleen is a treacherous organ in traumatic cases. Delayed rupture may occur after 48 hours with disastrous results.

6) Abdominal injuries in which there has been penetration demand surgical intervention as soon as the general condition of the patient permits. If continuing hemorrhage is suspected, delay in surgical intervention must not be tolerated.

7) Thoraco-abdominal incisions should be used more often in repair of large liver lacerations. Individual suture ligation or arteries, veins and bile ducts is of great import.

8) Ileus can be an annoying complication after severe trauma to the chest, abdomen, or pelvis.

9) In our emergency management of these cases several surgeons often work as a team, since the urgency of the situation demands the knowledge and application of more than one specialty group, viz., orthopedics, neurosurgery, urology, plastic surgery, thoracic, and general surgery.

DEGENERATIVE ARTERIOPATHY WITH PULMONARY HYPERTENSION: A REVISED CONCEPT OF SO-CALLED PRIMARY PULMONARY HYPERTENSION

THOMAS N. JAMES, M.D.*

The etiology of so-called "primary" pulmonary hypertension is a current medical enigma. It is not enigmatic for lack of attention, for there are a number of current theories, capably reviewed by Dresdale[1,2] and McGuire[3]. One of the popular theories attributes the arterial histopathology to long-standing arterial spasm[2,4,5] the cause of which is unexplained. Another theory suggests repeated embolization, based on the finding of apparent emboli in the small vessels of the lung at necropsy[4-15], but a consistent demonstration of a source for such embolization has not been presented.

Although inexorably down hill, the clinical course of this disease is of considerable interest. One of the features which has attracted study is the frequency of syncope, often as a presenting symptom[15-34]. Most explanations regarding this syncope have been unsatisfactory, principally because they have failed to account for the infrequency of syncope in patients with equal degrees of pulmonary hypertension secondary to some demonstrable cause.

Following the recent demonstration of vascular lesions in the sinus node and AV node of three patients with "primary pulmonary hypertension," it was postulated that these lesions are the mechanism of both the frequently observed syncope and sudden death[34]. Perhaps more important than a possible explanation of the mechanism of syncope and sudden death in these cases, however, is the fact that the vascular lesions observed in the sinus node and AV node were in systemic arteries. This makes the theory that the similar histopathology in the pulmonary arteries is secondary to pulmonary hypertension less tenable.

An investigation of other systemic arteries in these three necropsied cases of so-called "primary" pulmonary hypertension has confirmed the observation that an unusual process involves the walls of their systemic as well as pulmonary arteries. For this reason the three cases being reported are diagnosed *degenerative arteriopathy with pulmonary hypertension,* which for the sake of brevity will be referred to as DAPH. The clinical and necropsy findings in all three fulfill the diagnostic criteria of so-called "primary pulmonary hypertension".

CASE REPORTS

Case 1. M. B., a 31 year old white housewife, was admitted to the Henry Ford Hospital on May 22, 1960, because of fainting spells. She was well until syncopal bouts began 6 months prior to admission, progressively increasing in frequency so at the time of admission they were occurring several times daily. All the episodes were preceded by palpitation. The first occurred during an emotional upset, but subsequent ones had followed progressively decreasing amounts of physical exertion. For two weeks prior to admission there was increasing cardiac failure and dyspnea.

*Division of Cardiovascular Disease.
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She had had four normal pregnancies, with healthy children aged 12, 10, 8 and 6 years. In 1954 the patient had tubal ligation, and in October of 1957 a hysterectomy. An uncle, aunt and the patient's mother had diabetes. Her father and four siblings were alive and well.

On examination the patient was small, pale and of ectomorphic habitus. There was no cyanosis nor clubbing; peripheral pulses were normal. The lungs were resonant, with good breath sounds and no rales. Cardiac rhythm was regular at 90 per minute; blood pressure was 100/80 mm. Hg. A left parasternal heave was present, with a systolic thrill in the left second intercostal space; the second pulmonic sound was markedly accentuated and split, being followed by a soft blowing diastolic murmur at the left sternal border. The liver edge was palpable at the costal margin and tender, but did not pulsate. There was no peripheral edema.

An electrocardiogram showed right ventricular and right atrial hypertrophy with sinus tachycardia. Other laboratory studies, including blood and urine sugar determinations, were normal. She was treated conservatively, discharged May 23, and requested to return in one month for cardiac catheterization.

On June 26 a right cardiac catheterization was done by Dr. E. H. Drake. Pressures in the pulmonary wedge position, main pulmonary artery and right ventricle were respectively (in mm. Hg) 10 mean, 110/45 and 115/5-20. A small amount of oxygen desaturation in the femoral artery blood was raised to normal when the patient breathed 100% oxygen. Oxygen content of blood from the pulmonary arteries and right heart was normal. A diagnosis of primary pulmonary hypertension was made and the patient was again discharged. Treatment included digitoxin, hydrochlorothiazide and reserpine.

Syncope continued to increase in frequency, and her terminal admission was August 19, at which time a new murmur of tricuspid regurgitation was present. Cardiac rhythm was regular at 100 per minute. On August 20 an examiner noted frequent premature beats, and that evening there was bigeminal rhythm; no electrocardiogram was made then but the one of August 19 showed normal sinus rhythm and was unchanged from the one of May. During the night of August 21 the patient was found dead in bed.

At necropsy both the right atrium and ventricle were dilated and hypertrophied. There were no cardiac septal defects nor extracardiac shunts. The cardiac valves were normal. A moderate degree of sclerosis was present in all arteries, including the coronary arteries (Fig. 6). The sinus node artery and AV node artery both arose from the right coronary artery at the usual sites[35,36]. There were no obvious mural thrombi on gross inspection (see below). There were no gross pulmonary emboli and no apparent peripheral sources for emboli.

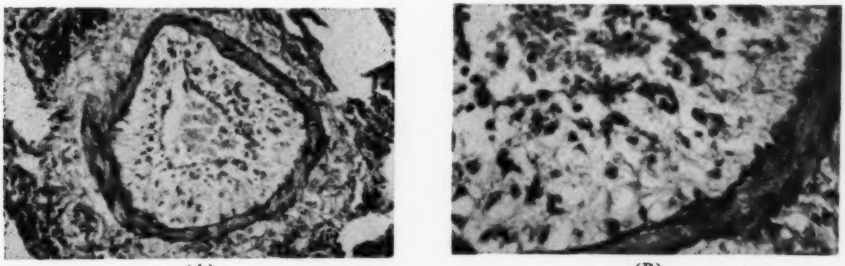
Histology of the pulmonary arteries is shown in Figs. 1-5; it is characteristic of "primary pulmonary hypertension". Notice the similarity to the arteriopathy of the systemic arteries shown from the heart and the adrenal gland (Figs. 7-15). The same arteriopathy was less frequently seen in the kidney, spleen, and liver.

Involvement of the sinus node artery was characterized by a focal granular degeneration of the tunica media, with some cyst formation and frequent hemorrhage (Fig. 7). An identical lesion was present in the AV node artery and some of the pulmonary arteries of this case (Figs. 3 and 10), and has also been described by others (Case 4 of Kuida, et al.[37]). Note the resemblance of the arteriopathy in the AV node and a larger ventricular artery (Figs. 6 and 12) to the prevalent lesion in Case 2 (Figs. 17-22, 25-30).

A variety of stains were employed in an effort to identify the amorphous material in the degenerated portion of the wall of the sinus node artery. It did not stain as collagen with the Goldner trichrome stain. It was not metachromatic with toluidine blue nor Alcian blue and was Schiff negative. It did not stain with Oil Red O. It had no affinity for iron. On staining with acridine orange the rim of the degenerating area fluoresced bright yellow under ultraviolet (Fig. 15), but the majority of the material did not fluoresce; there was no fluorescence with acriflavine, nor with any of the non-fluorochromatic stains. The material was not basophilic for hematoxylin.

Similar histochemical analysis of the intimal proliferation in the pulmonary arteries demonstrated that this process was Schiff positive, stained with Alcian blue and with the Rinehart-Abul Haj colloidal iron stain, was not fluorescent with any of the above stains, was not metachromatic with toluidine blue, and had no basophilic character. This material was likely an acid mucopolysaccharide. The endothelial *proliferative* process did not contain elastic fibers.

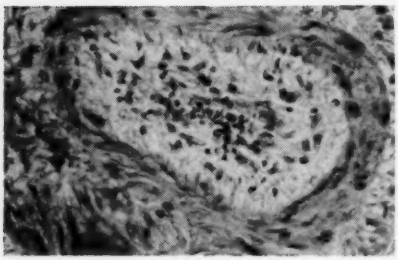
The old organized mural thrombus shown in Fig. 16 is directly underneath the sinus node and occupies the antrum atrii dextri of the crista terminalis, a site which Söderström[38] has indicated is a frequent one for atrial thrombosis. It is not difficult to see how such a thrombus may be overlooked, as it was in the gross study of this case.



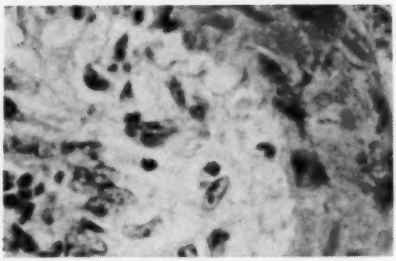
(A) (B)

Figure 1

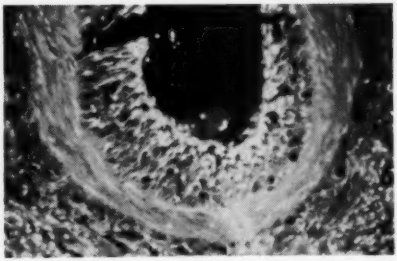
Small pulmonary artery from Case 1; note the remarkable intimal endothelial proliferation and the hypoplastic tunica media. Verhoeff-Van Gieson stain with magnification in *A* X64 and in *B* (same artery) X160.



(A)



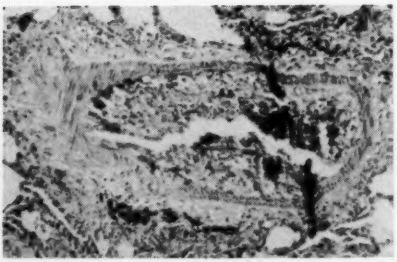
(B)



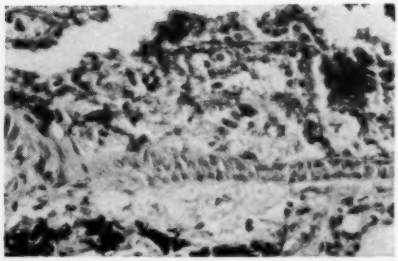
(C)

Figure 2

Another small pulmonary artery from Case 1, with the smudgy degeneration of the tunica media well shown under high power (A and B). Goldner trichrome stain with magnification in *A* X102 and in *B* (same artery) X256. *C* shows a similar artery; acridine orange stain, UV light, BG-12 filter, X64.



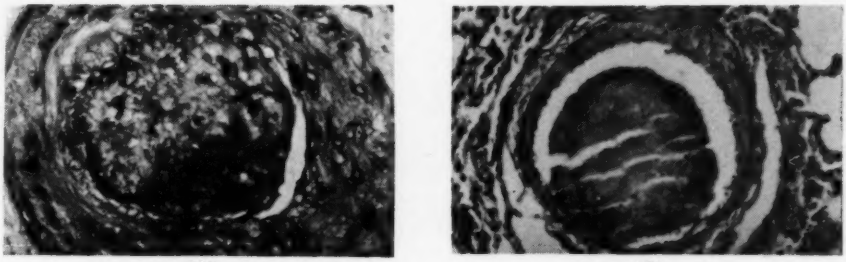
(A)



(B)

Figure 3

Small pulmonary artery from Case 1, demonstrating intramural degeneration and hemorrhage identical to that seen in the sinus node artery (Fig. 7) and AV node artery (Fig. 10). Goldner stain with magnification in *A* X40 and in *B* (same artery) X102.



(A) (B)

Figure 4

Two small pulmonary arteries of Case 1 with emboli. Note the distention of the vessels and the absence of attachment of the emboli to the wall or of any cellular reaction in the wall. *A* is stained with toluidine blue, X160; *B* is stained with Verhoeff-Van Gieson, X64.

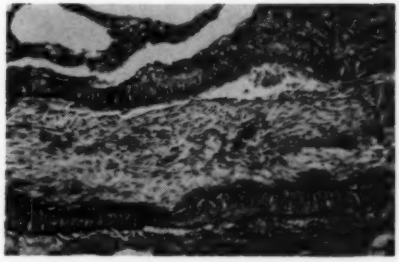
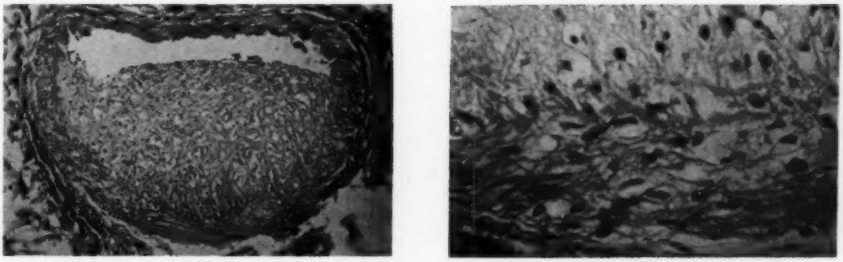


Figure 5

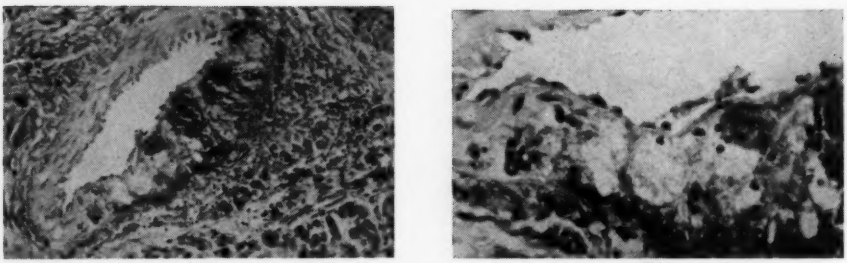
A longitudinal section of a small pulmonary artery from Case 1, demonstrating the intimal proliferation and thin tunica media; Verhoeff-Van Gieson, X64.



(A) (B)

Figure 6

A left ventricular coronary artery from Case 1, exhibiting an "atheroma"; Goldner stain. Magnification in *A* is X40 and *B* (same artery) X160. Cystic degeneration of the tunica media is apparent at the higher power.



(A) (B)

Figure 7

The sinus node artery from Case 1, with some extravasation of blood into the node. The granular compartmented degeneration with hemorrhage is well seen at the higher power. Goldner stain with magnification in *A* X64 and in *B* (same artery) X160.

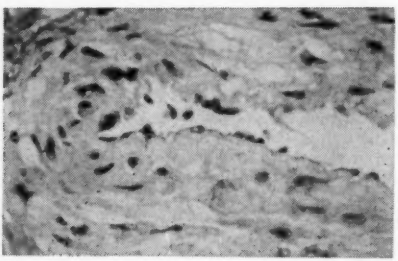
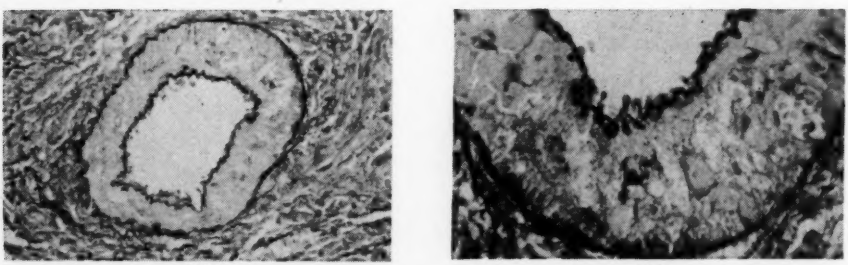


Figure 8

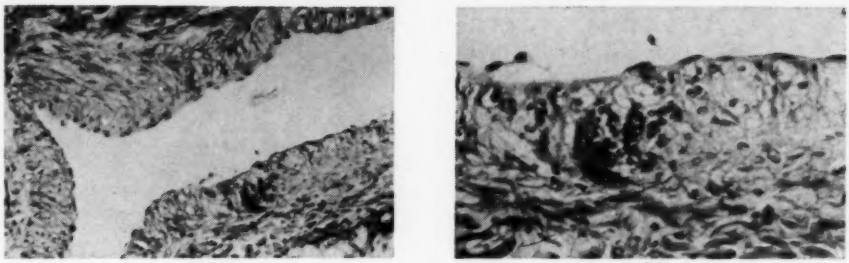
A section through tne sinus node artery of Case 1 taken 16 mm. from the section in Fig. 7. The degeneration in the tunica media is discernible at this magnification (X160) but is less apparent at lower power. Goldner trichrome stain.



(A) (B)

Figure 9

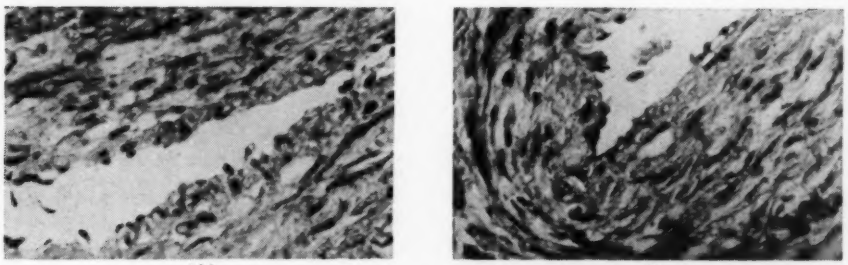
A section through the sinus node artery of Case 1 taken 4 mm. from the one in Fig 7. The internal and external laminae are intact, but the degeneration of the tunica media is obvious. Verhoeff-Van Gieson stain with magnification in *A* X64 and in *B* (same artery) X160.



(A) (B)

Figure 10

Artery to the AV node from Case 1, showing focal intramural hemorrhage and degeneration similar to that of the sinus node artery (Fig. 7) and a pulmonary artery (Fig. 3). Goldner stain with magnification in *A* X64 and in *B* (same artery) X160.



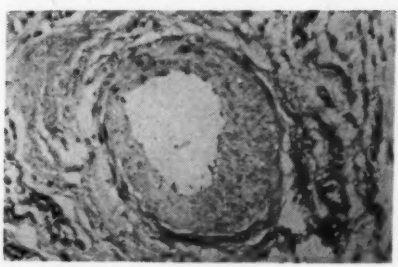
(A) (B)

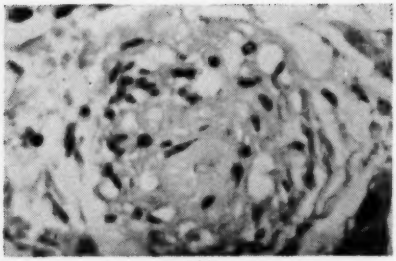
Figure 11

Two views of another portion of the AV node artery in Case 1, showing cystic degeneration of the tunica media. Goldner trichrome stain magnification in both *A* and *B* X160.

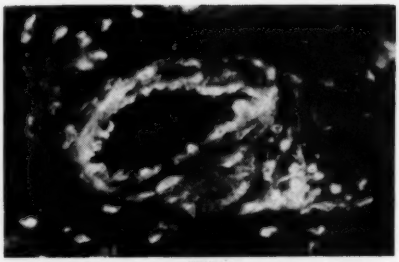
Figure 12

A third section through the AV node artery of Case 1, showing still a different arteriopaphy (although the same artery) from Figs. 10 and 11; the lesion here is primarily intimal proliferation and is identical to the predominant lesion in Case 2. Note the process here begins to comprise the lumen of the artery. Goldner stain, X64.





(A)



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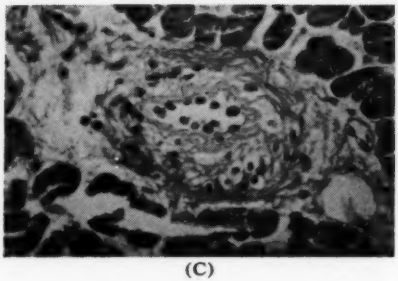


(C)

Figure 13

Three other abnormal myocardial arteries from Case 1. *A* is from the right atrium, showing an obliterative mixed intimal and medial proliferation; Goldner, X256. *B* is also from the right atrium, with primarily medial degeneration; acridine orange stain with ultraviolet light and BG-12 filter, X160. *C* is from the right ventricle, showing cystic medial degeneration; Goldner, X160.

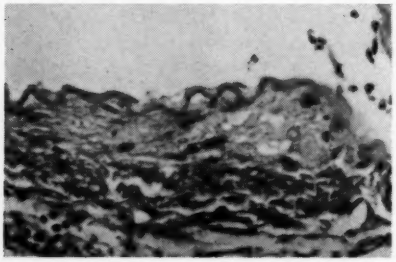
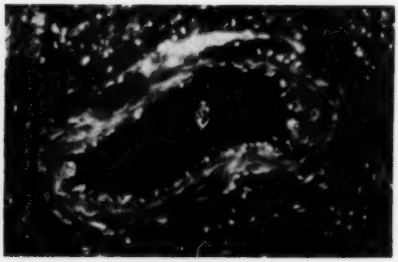
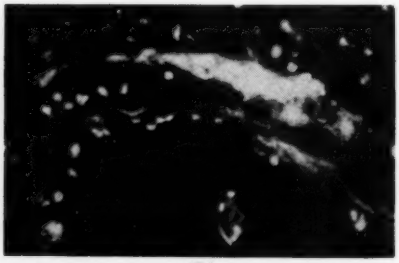


Figure 14

The wall of an adrenal artery from Case 1, showing granular and cystic degeneration; Goldner trichrome, X160.



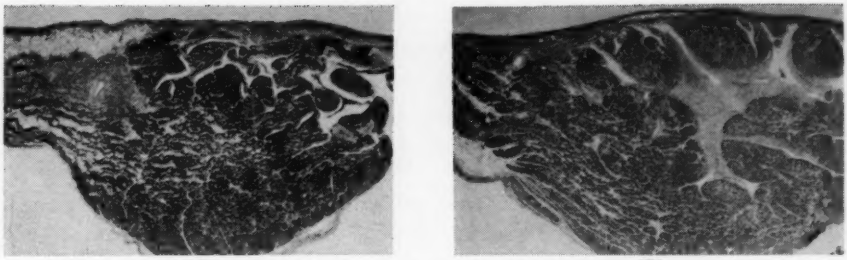
(A)



(B)

Figure 15

A section of the sinus node artery from Case 1 taken near that in Fig. 7; note the fluorescent rim in the area of maximal mural degeneration. Acridine orange stain with ultraviolet light and BG-12 filter; *A* X82, *B* (same artery) X160.



(A) (B)

Figure 16

Comparable sections of sinus nodes showing the vacant antrum atrii dextri of the crista terminalis in the normal (*A*) but the presence of an old organized mural thrombus in Case 1 (*B*). Both Goldner stain, X4. The sinus node can be seen about the artery in the upper left corner of each photomicrograph, near the epicardium; in *B* a focus of hemorrhage is visible in the wall of the artery, but the extensive infarction of the node which was present is not visible at this power.

Case 2. E. B., a 35 year old white unmarried schoolteacher, was delivered at the Henry Ford Hospital October 31, 1924; her mother's pregnancy and the delivery were uncomplicated. Except for one examination for a minor feeding problem in infancy, the patient was next seen July 6, 1950, complaining of weakness of both legs present 4 months. She also described coldness and numbness of the fingers on contact with cold for 3 years. Following hospitalization and extensive study, the final diagnoses were post-influenzal asthenia and mild Raynaud's phenomenon. In the review of symptoms she incidentally mentioned that she had been fainting about 3 or 4 times a year, usually in church, for the past 4 years. She had no other cardiac complaints and physical examination of the heart was normal. Erythrocytic sedimentation rate was 45 mm./hr., but other laboratory studies were normal. No electrocardiogram was made.

In January of 1958 she again complained of weakness beginning after "flu," followed by exertional dyspnea, orthopnea and the development of visible pulsations in the neck. After being ill for one month she was admitted to another hospital for two weeks, where she was told her heart was enlarged and sent home to rest. When her condition continued to deteriorate she returned to the Henry Ford Hospital and was admitted April 2, 1958.

On this terminal admission peripheral cyanosis and orthopnea were noted. The blood pressure was 105/60 mm. Hg but the sounds were faint. The femoral pulse was regular at 120 per minute; the radial pulses were difficult to palpate. The lungs were resonant and there were no rales. There was a left parasternal heave and the second pulmonic sound was accentuated. No murmurs were audible. Pulsations were present in the jugular veins; the liver was enlarged but not tender; there was moderate pretibial edema.

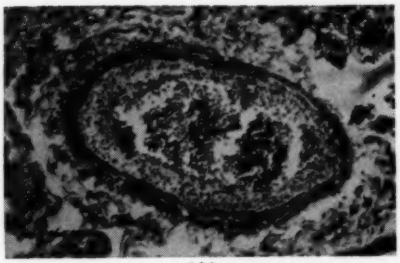
Except for a slight neutrophilia the blood studies were normal; repeated LE cell preparations were negative. There were some granular casts and 2+ albumin in the urine. At fluoroscopy there was marked cardiac enlargement and cardiac pulsations were virtually absent. An electrocardiogram on several occasions revealed right ventricular hypertrophy and sinus rhythm.

The patient remained afebrile throughout her hospital course. Two aspirations from the pericardium yielded 80 cc. of clear fluid and 165 cc. of serosanguinous fluid; these were negative for LE cells, and cultures were sterile. Despite conventional therapy for cardiac failure, this continued to increase, with the exception of pulmonary edema, from which she remained free. Ascites appeared and increased. On the 21st hospital day (April 23, 1958) she became more cyanotic, apprehensive, and expired quietly.

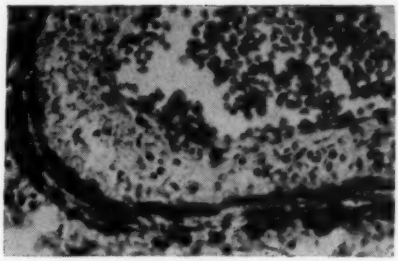
At necropsy there was a small amount of serosanguinous pericardial fluid. The right ventricle and right atrium were dilated and hypertrophied. Near the apex of the right ventricle there was a mural thrombus with central liquefaction. The arteries to the sinus node and AV node both arose from the right coronary artery. The cardiac valves and septa were normal and there was no arteriovenous shunt. No peripheral thrombi were found and no atrial thrombi were recognized on gross examination (see below).

The histologic appearance of the pulmonary arteries was characteristic of "primary pulmonary hypertension" (Figs. 17, 18). Note their resemblance to the arteriopathy present in the adrenals, brain, spleen, liver, kidneys and heart (Figs. 19-30). Prevalence of the systemic arterial lesions was marked in the adrenals, where virtually all the arteries were involved, and in the heart, especially the right atrium and sinus and AV nodes; in other organs the lesions had to be sought. The special similarity of the lesions in the adrenals, the heart and lungs is impressive. Hemorrhage in the tunica media is seen in Fig. 24, similar to that of the sinus node artery and some pulmonary arteries of Case 1. Histochemical studies in this arteriopathy gave the same negative results as in Case 1. There was no significant fluorescence with any of the stains studied.

As in Case 1 an antemortem thrombus was present underneath the sinus node (Fig. 31), and was not recognized on gross inspection. In discussing such thrombi, Söderström indicated that even though the portion of damaged atrial myocardium bordering on these sinuses in the crista terminalis was very small, the ensuing thrombosis usually filled most of the sinuses[38]. The possible role of emboli from such thrombi relative to both pulmonary hypertension and pulmonary arteriopathy in DAPH is the subject of later discussion.



(A)



(B)

Figure 17

Two views of the same pulmonary arteriole from Case 2, showing the typical intimal proliferation and the hypoplastic tunica media, visibly degenerated at the higher magnification. Goldner trichrome; *A* X64 and *B* X160.

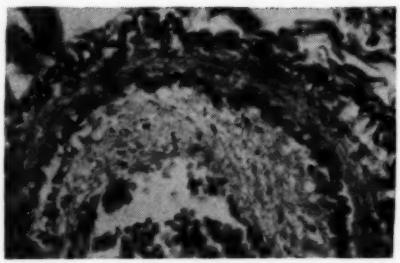


Figure 18

Another small pulmonary artery from Case 2, showing the same intimal proliferation and medial degeneration as Fig. 17, plus the intact internal and external laminae. Verhoeff-Van Gieson, X160.

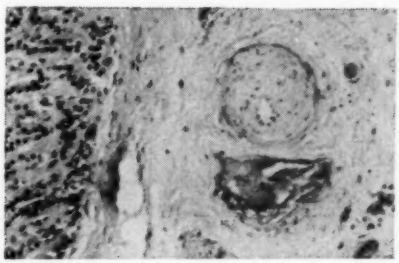
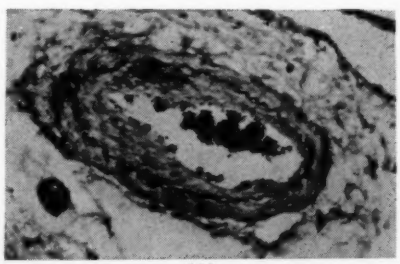
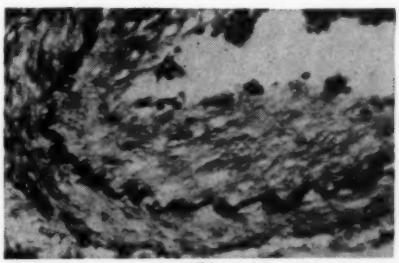


Figure 19

An adrenal artery from Case 2, with obliterative intimal proliferation. Hematoxylin and eosin, X64.



(A)

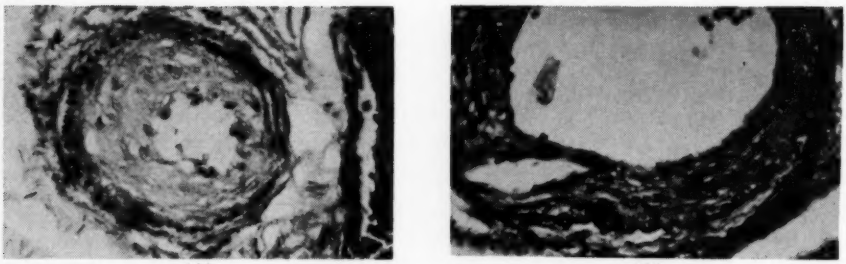


(B)

Figure 20

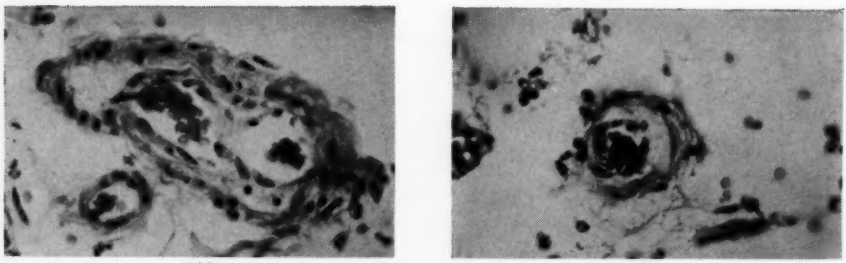
Another adrenal artery from Case 2, showing the same pathology as the pulmonary arteries in Figs. 17 and 18. The basic similarity of the arteriopathy in all the organs of each of the three cases soon becomes apparent. Verhoeff-Van Gieson; *A* X64, *B* (same artery) X160.

Figure 21
Adrenal artery from Case 2; note the similarity of this artery to those from the liver (Fig. 25) and kidney (Fig. 26). Hematoxylin and eosin, X64.



(A) (B)

Figure 22
Two other adrenal arteries from Case 2, showing the basic pattern of intimal proliferation and medial degeneration or hypoplasia. Verhoeff-Van Gieson, X160 in both *A* and *B*.



(A) (B)

Figure 23
Two small arteries from the brain of Case 2, with granular focal degeneration in the tunica media. Both *A* and *B* with Goldner stain at X160.

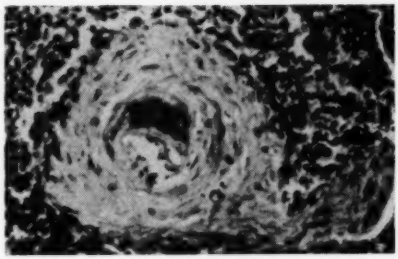


Figure 24
Small artery in the spleen of Case 2, with mural hemorrhage. Goldner, X160.

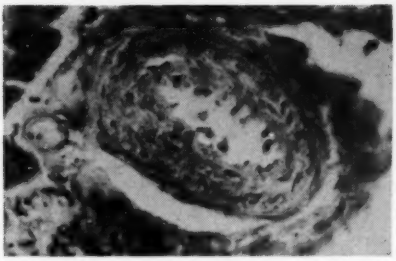


Figure 25
Intimal proliferation and medial hypoplasia in a small artery in the liver in Case 2. Goldner, X160.

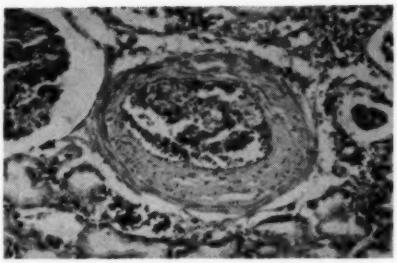
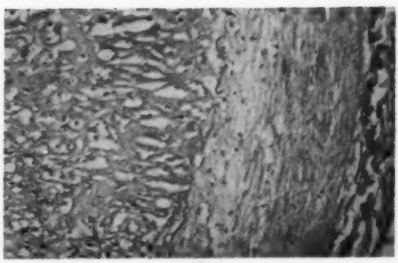


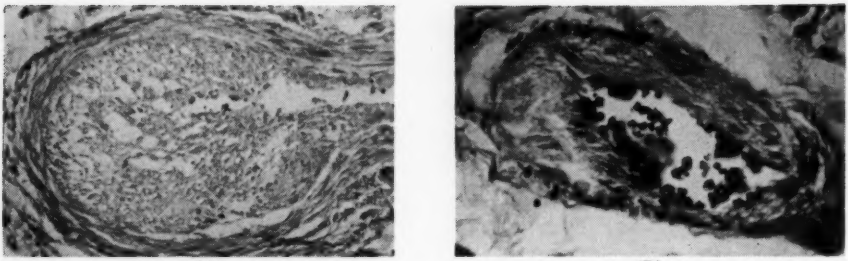
Figure 26
The same basic arteriopathy as in the other organs, shown here in the kidney of Case 2. Goldner, X64.

(A)



(B)

Figure 27
Two views of a section of the left coronary artery in Case 2, with an "atheroma." Goldner stain; *A* X10, *B* (same artery) X64.



(A) (B)

Figure 28

Two other coronary arteries from Case 2, *A* showing remarkable intimal proliferation and *B* smudgy medial degeneration. *A* is a branch of the right coronary artery in the eustachian ridge of the right atrium, Goldner, X64; *B* is a branch of the left coronary artery in the obtuse margin of the left ventricle. Goldner, X160.

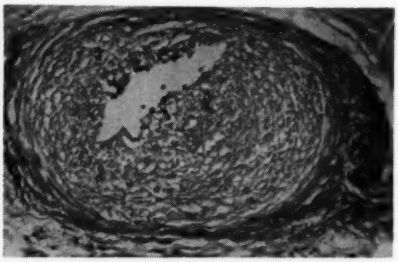
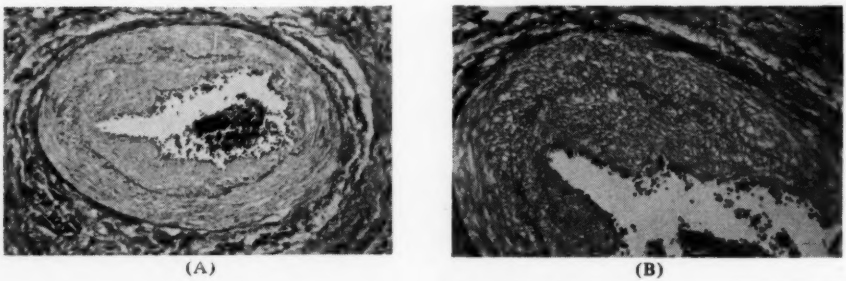


Figure 29

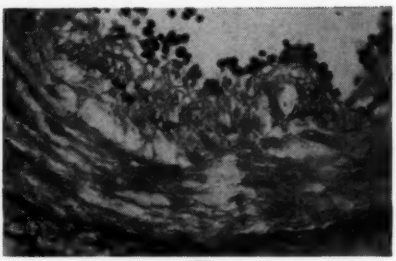
The artery to the AV node in Case 2, with the lumen virtually occluded by intimal proliferation. Goldner, X82.



(A) (B)

Figure 30

The sinus node artery from Case 2, showing the same arteriopathy as the artery to the AV node. All 3 views are of the same artery, Verhoeff-Van Gieson stain with *A* X40, *B* X64 and *C* X160. In *B* there is a gross defect in the tunica media which has been replaced by the intimal proliferative process; see text for a discussion of this relationship, and its applicability to the pathogenesis of systemic atherosclerosis. In *C* the moth-eaten appearance of the degenerating tunica media is well shown.



(C)

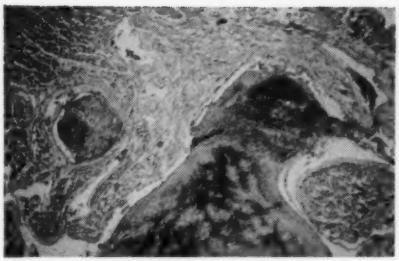


Figure 31
Antemortem mural thrombus in the antrum atrii dextri of the crista terminalis of Case 2, directly underlying the sinus node. Goldner, X10.

Case 3. J. M., a 29 year old white married housewife and mother of two children (ages 4 and 7) was referred to the Henry Ford Hospital from Grand Rapids by Dr. Noyes L. Avery, Jr. She was admitted May 9, 1958, with complaints of dyspnea and syncope which began 8 months previously. In October of 1957 she had been catheterized by Dr. Avery and the pressures in the wedged (pulmonary) position, the pulmonary arteries and right ventricle were respectively (in mm. Hg) 4 mean, 120/50 and 120/12. Right atrial pressure was 16/8 and the pulse contour suggested tricuspid valvular regurgitation. Oxygen contents were all normal. The diagnosis of primary pulmonary hypertension was made.

On admission to Henry Ford Hospital the patient was weak, orthopneic and slightly cyanotic. Blood pressure was 124/90 mm. Hg and cardiac rhythm was regular. There was a left parasternal heave and a blowing diastolic murmur along the left sternal border, with a loud systolic murmur and thrill in the same area. The second pulmonic sound was loud and split. There were no rales, but resonance and breath sounds were diminished at the right lung base. The neck veins were full and pulsating. Ascites was present.

NPN on admission was 70 mg. percent; there were granular casts and albumin in the urine. Repeated LE cell tests were negative. Other blood studies were normal. An electrocardiogram showed right ventricular hypertrophy and sinus rhythm.

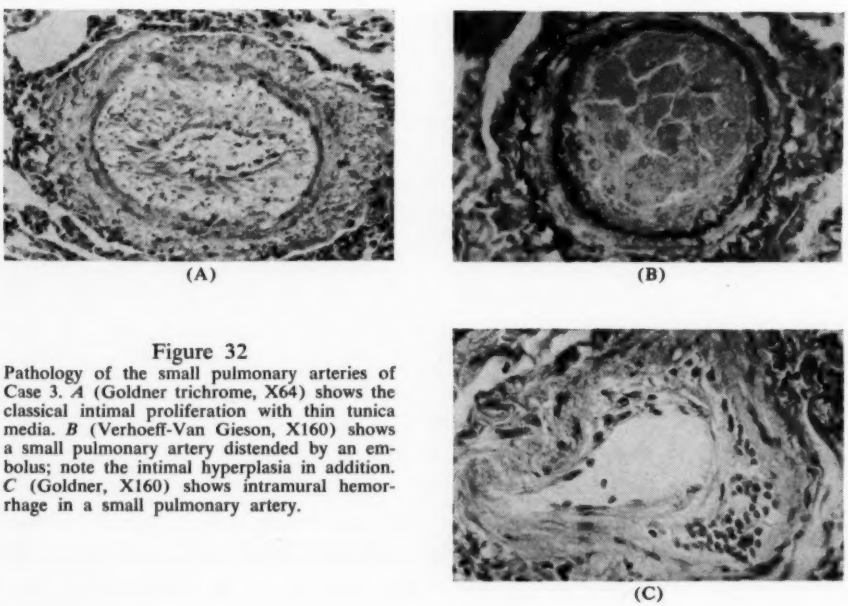
Her course in the hospital was marked by increasing weakness, oliguria and increasing right heart failure. She expired quietly on the fourth hospital day.

At necropsy there was dilatation and hypertrophy of the right atrium and right ventricle; the valves and septa were normal; mural thrombi were adherent to the endocardium of the right atrium near the sinus node, and the left ventricle. No arteriovenous shunt and no peripheral thrombi were found. There was marked gross atherosclerosis of the pulmonary arteries. The sinus node artery arose from the left circumflex coronary artery while the AV node artery arose from the right coronary artery. Congestion was present in the lungs and all the abdominal viscera.

Histology of the pulmonary arteries was characteristic of "primary pulmonary hypertension" (Fig. 32). In addition to the typical intimal endothelial proliferation and hypoplasia of the tunica media of the pulmonary arteries, there was an unusual number of emboli present in the small arteries, though none were grossly identified. Hemorrhage in the tunica media similar to that of Cases 1 and 2 is seen in Fig. 32 C.

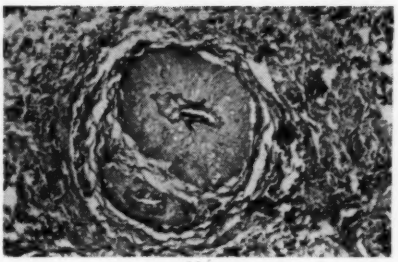
Although the primary feature of the arteriopathy in the sinus node was a non-specific proliferation of the tunica media (Fig. 33), focal degeneration and hemorrhage of the media, similar to that of Case 1, was also seen in other portions of the sinus node artery (Fig. 34). Other examples of the systemic arteriopathy are shown from the AV node, left and right ventricles, spleen and adrenal glands (Figs. 35-40). Similar but much less frequent lesions were seen in the kidney and liver. The medial hypertrophic process in the sinus node artery stained as collagen rather than muscle on the Goldner trichrome stain.

The antemortem thrombus in the right atrium was recognized at gross inspection in this case. As in the other two cases it was attached to the endocardium beneath the sinus node and filled the anterior sinuses of the crista terminalis (Fig. 41). It is the logical source for the emboli observed in the pulmonary arteries.

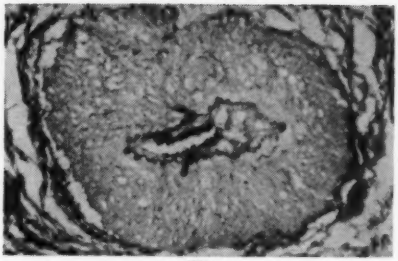


(A) (B) (C)

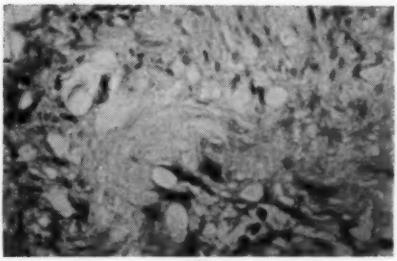
Figure 32
Pathology of the small pulmonary arteries of Case 3. *A* (Goldner trichrome, X64) shows the classical intimal proliferation with thin tunica media. *B* (Verhoeff-Van Gieson, X160) shows a small pulmonary artery distended by an embolus; note the intimal hyperplasia in addition. *C* (Goldner, X160) shows intramural hemorrhage in a small pulmonary artery.



(A)



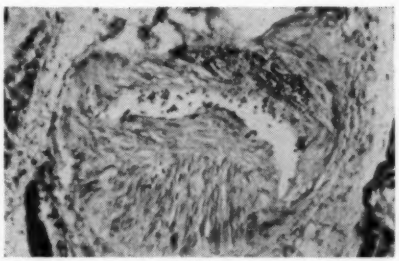
(B)



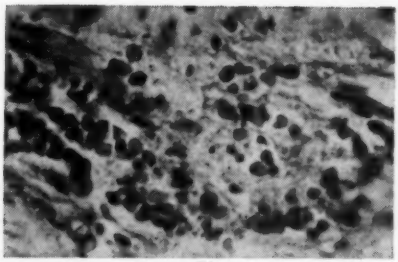
(C)

Figure 33

Three views of a section through the sinus node artery of Case 3. The predominant pathology here is a bizarre medial hyperplasia which virtually obliterates the lumen of the artery; there is some superimposed intimal proliferation visible inside the internal elastic lamina. This type of process sometimes occurs in the lungs, where it might be interpreted as "medial hypertrophy". It is clear from the higher power view in *C* that this is not a normal smooth muscle hypertrophy. Verhoeff-Van Gieson; *A* X40, *B* X82 and *C* X205.



(A)



(B)

Figure 34

An oblique section through the sinus node artery of Case 3, taken 12 mm. from the section of the same artery shown in Fig. 33. Here there is intramural degeneration and hemorrhage very similar to that in the sinus node artery of Case 1. Goldner trichrome stain; *A* X64 and *B* (same artery) X319.

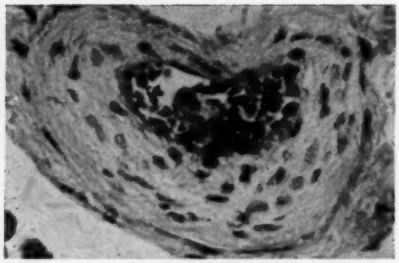


Figure 35
AV node artery of Case 3, showing intimal proliferation in which the nuclei are plump; the lumen of the artery is compromised. Goldner, X160.

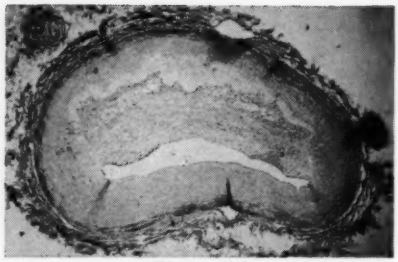
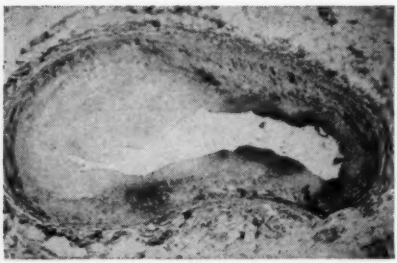
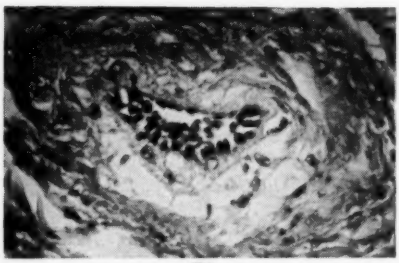


Figure 36
The left coronary artery of Case 3, showing an "atheroma" which is very similar to the coronary arteries of Cases 1 and 2. Goldner trichrome stain, X10.



(A)



(B)

Figure 37
The main right coronary artery *(A)* and a right ventricular branch (*B*) from Case 3; note the "atheroma" with medial thinning in *A*. There is cystic medial degeneration in the right ventricular branch. Goldner stain; *A* X10 and *B* X160.

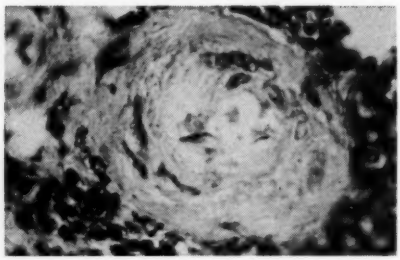
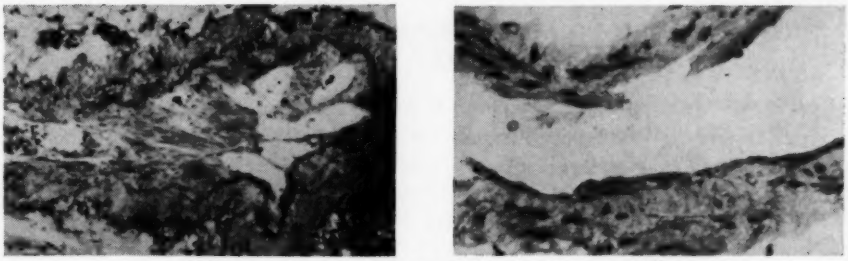


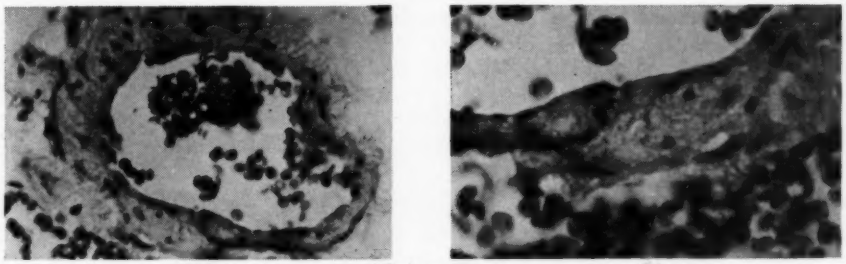
Figure 38
Granular medial degeneration in a small artery in the spleen of Case 3. Goldner, X319.



(A) (B)

Figure 39

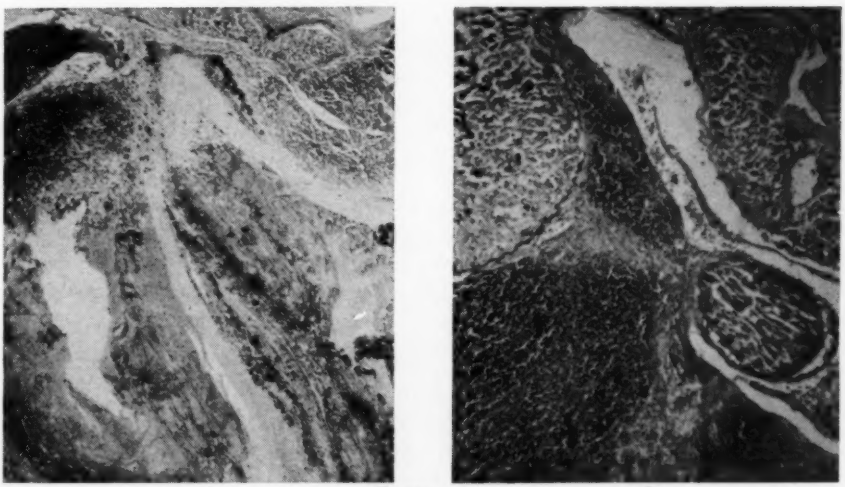
Adrenal arteriopathy in Case 3. *A* shows a bizarre intimal hyperplasia with a disorganized tunica media; Verhoeff-Van Gieson, X64. *B* shows granular degeneration and fragmenting of the tunica media; Goldner, X160.



(A) (B)

Figure 40

Another adrenal artery in Case 3, showing pathology similar to Fig. 39B. Goldner trichrome stain; *A* X160 and *B* (same artery) X319.



(A) (B)

Figure 41

Two views of the antemortem mural thrombus of Case 3, attached to the endocardium directly under the sinus node. *A* Goldner stain, X10; *B* Verhoeff-Van Gieson, X25.

CRITERIA FOR THE DIAGNOSIS OF DEGENERATIVE ARTERIOPATHY WITH PULMONARY HYPERTENSION

There is little question that the cases of "primary pulmonary hypertension" recorded in the literature represent a heterogenous group etiologically, a point emphasized by others[3,39,40]. A number of experienced observers have been impressed, however, with the frequency of similarities in one particular group, characterized as being represented by a young multiparous female with frequent bouts of syncope, clinical and laboratory evidence of marked pulmonary hypertension, absence of an arteriovenous shunt inside or outside the heart, absence of an apparent peripheral source for pulmonary emboli, and ultimately a relentless downhill course due to right ventricular failure[31,41]. It seems likely that this is a "pure" group, although as will be discussed later there are undoubtedly closely related and overlapping groups.

The conventional clinical and antemortem laboratory criteria for diagnosis of "primary pulmonary hypertension" (or DAPH), briefly summarized below, have been the subject of extensive description[1-3, 16-34] and do not therefore require fuller development here. They are as follows:

1. The patient is characteristically a young multiparous female.
2. Syncope is a frequent presenting symptom and usually becomes an increasing problem as the disease progresses; as a corollary, death is often sudden.
3. It is unusual to see cyanosis and peripheral edema early in the disease but these may appear in later stages.
4. Syncope, chest pain, and dyspnea commonly follow exertion, and as the disease progresses they occur with less and less stress.
5. Physical findings vary with the stage of the disease; early they are limited to evidence of right ventricular enlargement and a pulmonary ejection type murmur, with right ventricular failure minimal or absent. Later, right ventricular failure progressively increases, and eventually regurgitant murmurs appear at both the pulmonic and tricuspid areas. Pulmonary congestion is rare.
6. Electrocardiograms demonstrate right ventricular and right atrial hypertrophy, which are also apparent radiographically; radiographs show no pulmonary congestion and no left atrial enlargement, but the central pulmonary arteries are enlarged.
7. On cardiac catheterization the pulmonary wedge pressure is normal but the pressure in the pulmonary arteries and right ventricle markedly elevated. There is no evidence of arteriovenous shunting and, until late in the disease, no peripheral oxygen desaturation. The increased hazard associated with cardiac catheterization in these patients has been well documented[27,28].
8. There is no evidence of a peripheral source for pulmonary emboli, no intrinsic non-vascular pulmonary disease, and no evidence of systemic

diseases known to be associated with arteriopathy, such as lupus erythematosus or scleroderma.

Gross findings at necropsy consist of right ventricular and right atrial hypertrophy and dilatation, and frequently grossly visible sclerosis of the larger pulmonary arteries. Thickened smaller arteries may be visible on the cut surface of the lung. Small emboli in the pulmonary arteries are commonly found, but larger emboli are not. There are no cardiac septal defects nor any valvular lesions, though the pulmonic and tricuspid valve rings may measure as slightly dilated. No peripheral thrombi are present. Though intracardiac thrombi have only seldom been reported[22,42], small intertrabecular thrombi in the right atrium and ventricle can easily be missed. Their presence in all three of the present cases, as well as their potential role in the pathogenesis of the pulmonary hypertension makes a careful *gross and microscopic* search for them in future cases important.

Microscopic findings reported in the past have been limited almost exclusively to those of the lungs. With few exceptions[40,43] histology of other arteries has not been reported, except in general terms as "negative". The frequency of emboli or thrombi in the smaller pulmonary arteries varies considerably in reported cases. Most of the pathological changes previously described in the smaller pulmonary arteries differ little in DAPH from those found in secondary pulmonary hypertension, but the extent of these changes is different. In DAPH remarkable intimal endothelial proliferation (Figs. 1, 2, 17, 18, 32A) is more frequent and more striking. Some consider a capillary proliferative ("glomoid") lesion highly suggestive[43]. But the really significant changes occur in the tunica media.

Bredt was among the first to be impressed by the medial degeneration seen in the pulmonary arteries in "primary pulmonary hypertension"[44]. Later, Gilmour and Evans[45] called attention to "aplasia or hypoplasia" of the media. Though Bredt as well as Gilmour and Evans postulated that the medial degeneration was the primary lesion and that pulmonary hypertension developed as a consequence, this postulate remained speculative and the prevailing current interpretation has been that the pulmonary arteriopathy is the consequence of the hypertension and not the cause of it. The present demonstration of a similar arteriopathy in normotensive systemic arteries of three patients with "primary pulmonary hypertension" now makes re-evaluation of this question necessary.

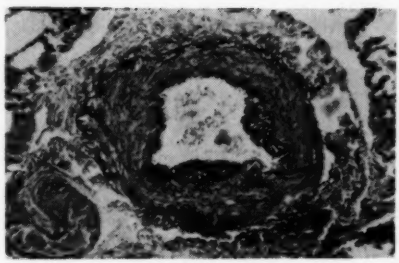
Instead of extensive hypertrophy of the smooth muscle of the tunica media of the pulmonary arteries, which is characteristic of most states associated with pulmonary hypertension, the arteries of the lungs of patients with DAPH show a singular lack of such response. When one searches carefully enough, a few vessels with medial hypertrophy may be found in DAPH, but these are exceptional and by far the prevalent lesion is the medial degeneration and hypoplasia. Conversely, on careful search of the lungs from cases of secondary pulmonary hypertension medial degeneration or hypoplasia is occasionally encountered, but again this is the exceptional lesion.

The medial degenerative changes are characteristic. Though these sometimes show small cyst formation, somewhat similar to Erdheims' cystic medial necrosis[48,49], they are more typically a ground-glass or finely granular degeneration of the smooth muscle fibers (Figs. 2B, 7B, 8, 9B, 30C). Significantly, there is no associated inflammatory cellular response. In other arteries a pyknotic degeneration of the nuclei of arterial smooth muscle occurs, perhaps as an earlier stage. Intimal hyperplasia or proliferation is usually associated with underlying medial atrophy or hypoplasia. In most areas both the internal and external elastic membranes remain intact, but occasionally the former becomes slightly frayed. All these lesions are segmental or focal, and serial sectioning demonstrates that the same artery may appear normal in one area but diseased further on[22,29].

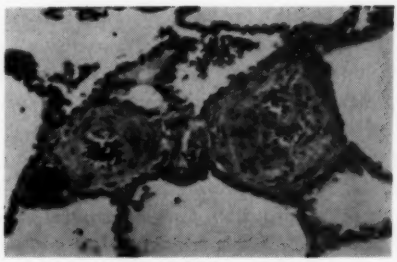
Since the specific arteriopathy in the three present patients differs in some arteries from others, one may consider whether the three patients all represent the same disease. For example some arteries show recent medial degeneration with hemorrhage while others show intimal proliferation and hyperplasia. Since all these lesions were present in one artery or another in all three patients, it is probable that they all are the same disease and that these variations in arteriopathy are more apparent than real.

In general the arteries with recent medial degeneration showed little intimal response, while those with extensive intimal response showed no recent disease in the tunica media, which was, however, usually atrophic or hypoplastic. It is reasonable to believe, therefore, that the medial changes are the initial ones and that the intimal response is secondary to this, the difference in histologic appearance thus depending on the time of examination in the natural course of the arteriopathy. Presuming the basic pathology is a generalized degenerative arteriopathy, one is still faced with the need for explaining the higher prevalence of the lesion in the lungs. This is the subject of later discussion.

One type of arterial medial response in DAPH may be confused with conventional medial hypertrophy seen in secondary pulmonary hypertension. The sinus node artery of Case 3 illustrates this process: note whereas the tunica media is tremendously "hypertrophied", this hypertrophic process does not include normal smooth muscle but is formed of relatively non-specific cells (Fig. 33). Contrast this to the hypertrophy of smooth muscle seen in the pulmonary arteries in secondary pulmonary hypertension (Fig. 42). Thus though a sort of medial proliferative process may be seen in arteries in DAPH, it is not a normal smooth muscle response to increased work. Possibly it is a non-specific fibroblastic reparative response[46] to smooth muscle degeneration in the tunica media. That it is not an unusual smooth muscle hypertrophy (e.g. longitudinal fibers) responding to the increased work demands of pulmonary hypertension is suggested by the presence of an identical process involving systemic arteries.



(A)



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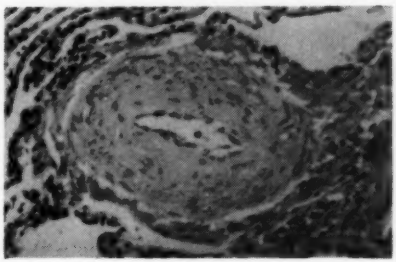


(C)

Figure 42

Smooth muscle hypertrophy in small pulmonary arteries of two patients with secondary pulmonary hypertension. *A* and *B* are from a 19 year old male with severe pulmonary hypertension and reversed flow in a patent ductus arteriosus; *C* is from a 29 year old female with severe pulmonary hypertension due to an interatrial septal defect. Contrast the relatively orderly smooth muscle hypertrophy here with the bizarre medial proliferation in Fig. 33. *A* Verhoeff-Van Gieson, X32. *B* Goldner, X82. *C* Goldner X82.

Of considerable importance in differentiating the arterial lesion of DAPH from other arteriopathies is the absence of any inflammatory cellular response to the degeneration. There are no giant cells nor a leukocytic response, which helps to distinguish it from most of the arteriopathies associated with the "collagen" diseases. In Case 3 there were a large number of mast cells around the arterial lesions of the lung, but not about the systemic arterial lesions; in the other two cases there were no more mast cells than normally seen in relation to blood vessels.

SIGNIFICANCE OF SYSTEMIC ARTERY INVOLVEMENT

Involvement of the arteries to the sinus node and AV node, which are systemic arteries, explains the syncope and sudden death which are commonly observed in "primary pulmonary hypertension". Others have occasionally noted involvement of various systemic arteries of patients dying with this disease, but have either interpreted it vaguely or could not be certain they were not coincidental lesions. This particular arteriopathy in the cardiac nodes must be accepted as an integral part of the basic disease process, since the clinical features it produces are accepted as an integral part of the basic clinical picture of "primary pulmonary hypertension".

In their discussion of the etiologic factors involved in "so-called primary pulmonary hypertension", Rawson and Woske[43] described one case in whom extrapulmonary arterial lesions were found; these consisted primarily of a perivascular cellular reaction and no details are given of the histology of the walls of these arteries. On the basis of this and the clinical symptoms of 3 other cases, plus a review of the symptoms of reported cases in the literature, they suggested that "primary pulmonary hypertension" may be related to the "collagen" group of diseases.

Although the arguments of Rawson and Woske are consonant with the concept of "primary pulmonary hypertension" as a systemic disease, the systemic arterial histopathology in their case was somewhat different from that observed in the three present cases. The similarity of the medial involvement in these three cases in both the *systemic* and *pulmonary* arteries, as well as its similarity to the process in pulmonary arteries described by Gilmour and Evans[45], Bredt[44], Wade and Ball[48], Kuida et al[37], and Berthrong and Cochran[47], suggests that this is a specific arteriopathy. There are probably cases of atypical collagen diseases with somewhat similar changes; these may represent intermediary or overlapping forms inevitably found in virtually all arteriopathies.[47-59]

There is little question that severe hypertension is often associated with necrosis of the tunica media, and the frequency of this phenomenon in malignant systemic arterial hypertension makes the previous presumption that such changes in the pulmonary arteries were also the consequence of abnormally elevated pressure plausible. Some of the changes observed in the media still quite likely are the consequence of hypertension, but the presence of similar changes in non-hypertensive systemic arteries makes it unlikely that hypertension is the only cause. It is possible that some of the arteriopathy in the lung involves bronchial (systemic) arteries rather than hypertensive pulmonary arteries.

PATHOGENESIS OF THE PULMONARY HYPERTENSION

Some substance present in the venous blood returning to the right heart and lungs has long been postulated as a possible cause of pulmonary arterial and arteriolar spasm eventually leading to irreversible vascular damage and right heart failure. The basic flaw in this theory has been the inability to identify such a substance in patients with the disease. The present findings do not refute this possibility, but they do indicate several previously unrecognized factors at least contributing to the hypertension.

One of these factors is the demonstration of a source for embolism to the lung. All three of the present patients had mural thrombi in the right atrium (Figs. 16, 31, 41); in two of the three cases the thrombus was not detected at the time of gross examination, but was demonstrated microscopically. Since various stages of injury were present in the sinus node of all three cases, varying from old fibrosis to recent hemorrhage, it seems reasonable to believe that this area is the site of repeated small infarctions and associated mural thrombosis. Repeated embolization of small fragments from these thrombi would be unlikely to produce clinically recognizable bouts of pulmonary embolism, but in association with a pulmonary arterial bed already diseased it could readily lead to a vicious cycle of events (Fig. 43). Repeated attacks of transient arrhythmias during the syncopal attacks could further contribute to right atrial stasis and thrombus formation.

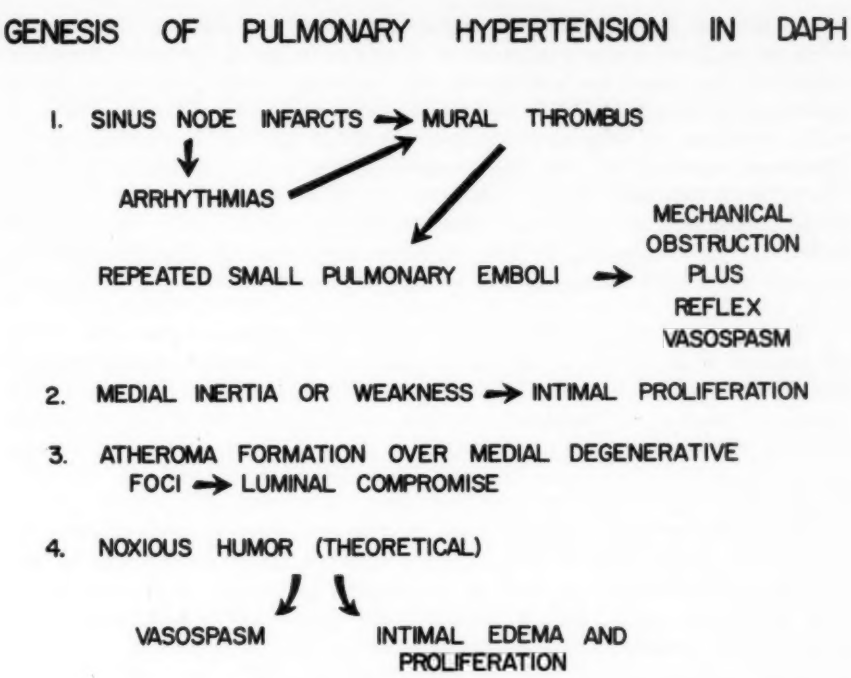

GENESIS OF PULMONARY HYPERTENSION IN DAPH
1. SINUS NODE INFARCTS → MURAL THROMBUS
ARRHYTHMIAS
REPEATED SMALL PULMONARY EMBOLI →
MECHANICAL OBSTRUCTION PLUS REFLEX VASOSPASM
2. MEDIAL INERTIA OR WEAKNESS → INTIMAL PROLIFERATION
3. ATHEROMA FORMATION OVER MEDIAL DEGENERATIVE FOCI → LUMINAL COMPROMISE
4. NOXIOUS HUMOR (THEORETICAL)
VASOSPASM
INTIMAL EDEMA AND PROLIFERATION

Figure 43
A schematic indication of the factors involved in production of pulmonary hypertension in DAPH.

Emboli from the endocardium beneath the sinus node may produce pulmonary hypertension in three ways. Mechanical obstruction is the first of these, but it is improbable it is a major factor because of the relatively small size of the initial mural thrombus as well as the lack of recognition of clinical episodes suggesting pulmonary embolism in these patients. More likely the small emboli have a more chronic effect by stimulating an arterial proliferative process demonstrable experimentally[6-9,14]. The third effect involves the possible release of serotonin from the mural thrombus as well as the small emboli from it; this substance has been demonstrated to be a powerful stimulator of pulmonary vasoconstriction[60, 61]. The latter effect is likely operative only while active thrombosis is present and capable of releasing serotonin from disintegrating platelets, but how often in the course of DAPH active mural thrombosis is occurring is unknown.

The seeming inadequacy or inertia of the pulmonary arterial media, as expressed by its failure to hypertrophy under the hypertensive stimulus, suggests another interesting hypothesis. If the tunica media which is later seen to degenerate is at an earlier stage simply weak, arteriolar dilatation may be expected to occur. According to LaPlace's law, the pressure in a hollow chamber (in this case the artery) is directly proportional to the tension in the chamber wall and inversely proportional to the radius of the chamber. Thus if the tension in the arterial wall decreased, in order

to maintain the same pressure the radius of the artery would have to decrease. Since the weakened media is incapable of doing this via spasm, the usual physiologic means, one may reason teleologically that the body takes another recourse by intimal endothelial proliferation.* That the intimal proliferation corresponds to points of medial hypoplasia, as observed by Gilmour and Evans and also in the three present cases, may mean that the body has attempted to heal a point of medial weakness. The beautiful concentric intimal proliferation so characteristic of DAPH may thus be the consequence of evenly distributed concentric medial weakness, and the focal "atheroma" the consequence of focal medial weakness. The possible relationship of the same hypothesis to pathogenesis of systemic atherosclerosis will be discussed later.

Demonstration that priscoline and acetylcholine are capable of transiently lowering the pressure in the pulmonary arteries of patients with "primary pulmonary hypertension"[2,62,63] suggests vasospasm is present, but it has not defined the cause of the vasospasm. Because of consideration regarding a neurogenic cause[2] thoracic sympathectomy has been attempted as treatment, but the patient did not improve[64]. More recently attention has been directed to the possible effect of serotonin, and its ability to produce a type of arteriopathy[65,66]; this seems an unlikely major factor because of the absence of other findings due to increased circulating serotonin seen in malignant carcinoid. Perhaps more suspicion should be directed at epinephrine and norepinephrin, particularly in view of the presence of adrenal arteriopathy in all 3 of the current cases. Though relatively weak pulmonary vasocontrictors, these catecholamines potentially have other effects on the pulmonary arterial wall which may lead to luminal obstruction; this will be developed more fully subsequently.

Whether vasospasm is due to some substance circulating in the blood or due to a neurogenic reflex, perhaps related to repeated small embolisms, there is no doubt that organic obstructive lesions are present in the majority of authentic cases of "primary pulmonary hypertension". One cause for these lesions is embolism, but for a more complete understanding of the disease it is necessary to define the nature of the primary degenerative changes in the arterial wall.

ETIOLOGIC POSSIBILITIES REGARDING THE DEGENERATIVE ARTERIOPATHY

Although the present findings do not exclude the role of a noxious venous substance in the pathogenesis of DAPH, it is not necessary to explain the disease on this basis. The pulmonary hypertension and clinical course can all be explained on the basis of the degenerative arteriopathy without the presence of such a substance. The medial weakness as a possible cause of intimal proliferation has already been discussed, and the manner in which this would contribute to hypertension if unchecked is apparent; the additional occurrence of repeated small emboli from recurring mural thrombosis near the sinus node would aggravate and accelerate this process, ultimately causing it to become self-propagating: the pulmonary hypertension producing more

*A lucid differentiation of *intimal elastic hyperplasia*, commonly seen in atheroma, and *intimal endothelial proliferation*, which is uncommon except in "primary pulmonary hypertension," is made by Brenner[39]. Both processes are seen in DAPH.

arterial medial damage which through intimal proliferation would in turn lead to more hypertension (Fig. 43).

What then are the possible causes of the degeneration in the tunica media? There is a resemblance to the medial lesions of Marfan's syndrome and many other heritable disorders of connective tissue[67,68]. In both diseases sudden death is a common phenomenon, and the possibility that it is sometimes due to involvement of the nodal arteries in Marfan's syndrome as well as in DAPH is now being investigated. Cystic medial degeneration occurs in the pulmonary artery in both diseases and ruptured dissecting aneurysm of the main pulmonary artery has been described in "primary pulmonary hypertension"[69-72]. The histology of the media of the aorta in the three present cases was normal, but this needs to be studied further in other cases of DAPH. Similarly the histology of the smaller pulmonary arteries needs to be examined in Marfan's syndrome, as well as in experimental lathyrism[73-75]. Recently vascular lesions were produced in swine fed a copper-deficient diet[76], an observation which deserves further study.

If a specific degenerative process of the tunica media of certain arteries is the primary pathology in DAPH, this may be either congenital or acquired. Much the same analysis regarding these possibilities may be applied to DAPH as to Marfan's syndrome[67,68]. Since a familial incidence of "primary pulmonary hypertension" has been observed[1,2,26], it will be important to determine if any relatives of patients with heritable disorders of connective tissue develop DAPH. Berthrong and Cochran suggested unexplained pulmonary hypertension in children may be associated with an increased incidence of congenital cardiovascular malformations (themselves not producing the pulmonary hypertension)[47]. If it is an acquired lesion, there has so far been no explanation of how it becomes acquired. It is possible there is a hereditary predisposition which, in combination with some process which would otherwise be innocuous, results in arterial medial degeneration in these susceptible patients.

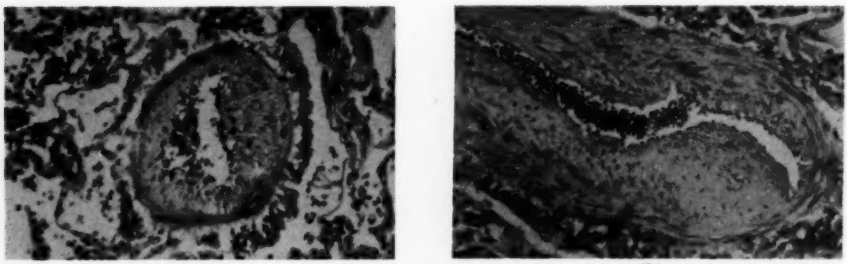
One possible means of acquisition of the arteriopathy which has impressed numerous observers is pregnancy. The primary problem with accepting pregnancy as the only process responsible for DAPH is the observation of the disease in women who have never been pregnant (Case 2, e.g.), as well as some acceptable cases in males[23,25,27]. The placenta or the gravid uterus as the possible source of a substance aggravating or even producing DAPH can only be accepted if there are also other causes. A number of other cardiovascular diseases seem equally obscurely related to menstruation and pregnancy[77-81].

That pregnancy and breeding have an influence on arteries is well documented. It has been demonstrated that experimental animals repeatedly bred are more susceptible to atherosclerosis than non-bred controls[82]. The occurrence of dissecting aortic aneurysm in pregnancy has been reported numerous times, and its possible relationship to hormonal derangements of pregnancy have been the subject of study[60,83,84]. Aortic dissection in experimental lathyrism is reported to be more frequent

in male than female animals[75], but the histology of the pulmonary arteries in these animals has not been reported.

Among endocrine secretions possibly responsible, one which has some experimental and clinical observations to support it is the thyroid stimulating hormone (TSH). It has recently been reported that the administration of high levels of TSH produces certain degenerative myxomatous changes in muscle[85] somewhat suggestive of the lesion in the tunica media of DAPH. These changes did not occur in control animals treated with thyroid hormone alone. Some years ago Kountz and Hempelmann reported the occurrence of dissecting aortic aneurysm in three consecutive patients with severe systemic hypertension treated with thyroidectomy[86], and convincingly argued that the thyroidectomy and dissection were related; these patients presumably developed elevated levels of TSH. Myxomatous degenerative changes in the heart and in the coronary arteries have long been recognized as commonly occurring in myxedema[87-89] and have been presumed to be related partly to elevated cholesterol levels; perhaps the TSH level in these patients is equally important.

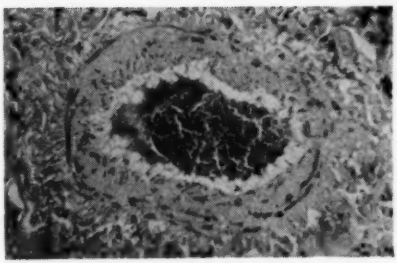
Also deserving consideration in the etiology of the degenerative arteriopathy are epinephrin and norepinephrin, which are capable of producing arterial lesions experimentally[90-92]. A very similar change is sometimes seen in the pulmonary arterioles of patients with pheochromocytoma (Fig. 44, 45). In all three of the present patients there was arteriopathy present in the adrenal glands, involving occasional arteries in Cases 1 and 3, but virtually all the adrenal arteries in Case 2 (Figs. 14, 19-22, 39, 40). Furthermore the process in the adrenal arteries was the same as the process in other systemic arteries and the pulmonary arteries in each case, as well as characteristic of lesions generally described in "primary pulmonary hypertension" in the lungs by others.



(A) (B)

Figure 44

Two small pulmonary arteries from the lungs of a young woman who died of pheochromocytoma. Note the similarity of the arteriopathy to many of the arteries in Cases 1, 2 and 3. *A* (Goldner, X64) shows intimal proliferation with a thin tunica media. *B* (Goldner, X64) shows focal intimal proliferation over an area of medial thinning.



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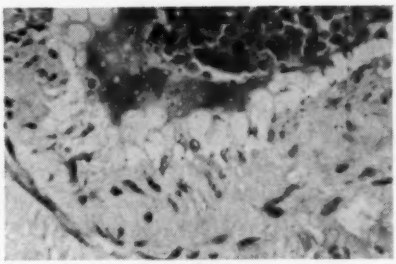

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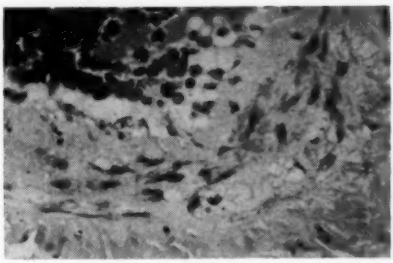


(C)

Figure 45
Pulmonary arteriopathy in another case of fatal pheochromocytoma, this in a middle-aged male. *A* and *B* are the same artery, Goldner stain, at X64 and X160; there is a small amount of intimal proliferation, with edematous endothelial cells overlying it, and with a disorganized and degenerate tunica media underlying it. Another artery with a similar process is shown in *C* (Goldner, X160), with the cystic changes in the tunica media clearly seen.

How often there is extensive adrenal arteriopathy in DAPH cannot be stated from observations in 3 cases. Since it was extensive in one of the three, however, the possible role of adrenal ischemia must be considered. During right heart failure which is common in DAPH, right atrial and peripheral venous pressure rises, and this must include pressures in the inferior vena cava and adrenal veins. Such venous hypertension coupled with the fall in aortic pressure which occurs would be further aggravated by luminal lesions in the adrenal arteries, thus suggesting that a disproportionate ischemia must occur in the adrenal glands, similar to that affecting the cardiac nodes, where the arteries are similarly involved. That adrenal ischemia is associated with increased elaboration of pressor agents has been suggested from experiments.[93] It is reasonable to presume, therefore, that an excessive amount of epinephrin or norepinephrin (or both) will reach the pulmonary arteries in patients with DAPH at least intermittently.

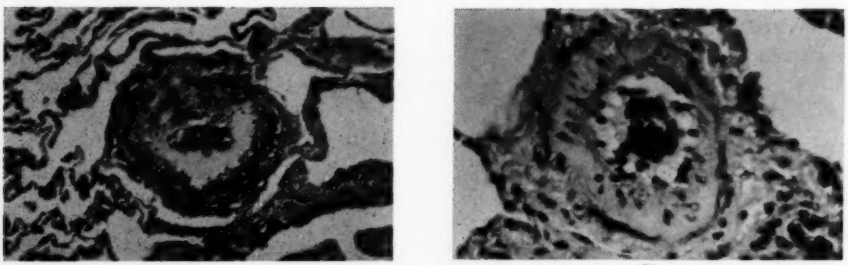
Since epinephrin and norepinephrin have been shown to be only weak pulmonary vasoconstrictors in animals and normal man[94-95], the presence of pulmonary arteriopathy in patients with pheochromocytoma is puzzling. The lesion illustrated in Fig. 45 suggests edema or slight proliferation of the intimal endothelium; there were in addition arteries with a moderate degree of smooth muscle hypertrophy and some cystic degeneration; occasional arteries resembling those of DAPH were also found (Fig. 44). Others have described extensive smooth muscle hypertrophy, as well as degenerative changes of various types in the tunica media of the pulmonary arteries of patients with pheochromocytoma[97,98]. The pressure in the pulmonary arteries of such patients has not been measured, so it is improper to presume that it is not elevated because of experimental observations in animals or in man without pheochro-

mocytoma. It is not known what the response of the arteries in "primary pulmonary hypertension" is to epinephrin and norepinephrin, though their hypotensive response to adrenolytic agents has been repeatedly observed[2,62,63].

Regarding the sexual predilection of DAPH, it has been shown that women's conjunctival arterioles become exquisitely sensitive to epinephrin during menstruation[99]. Conceivably the preponderance of DAPH in women in the child-bearing age is related to this observation. It may be well to study the response of the conjunctival arterioles to various pressor agents in patients with DAPH.

Despite these intriguing implications, it is unlikely epinephrin or norepinephrin is the major factor in DAPH. Absence of systemic hypertension indicates there is no increase in total circulating catecholamines. Presence of systemic arterial lesions indicate the arteriopathy probably occurs without abnormal blood pressure elevation. The evidence suggests that epinephrine or norepinephrin may contribute intermittently to the pulmonary hypertension and arteriopathy, however, and this possibility deserves further investigation.

Recently attention has been directed to serotonin as a possible etiologic factor in "primary pulmonary hypertension", its powerful vasospastic effect on the pulmonary circulation being well known. Administration of serotonin has produced a pulmonary arteriopathy in rabbits but failed to do so in rats.[66] Since nature has provided a specific human example of the effect of excessive secretion of serotonin in the malignant carcinoid syndrome, and since its clinical and pathologic characteristics[100,101] are considerably different from those of DAPH, it seems unlikely that serotonin is a significant factor in the latter. Resemblance of pulmonary arteriopathy in one case of malignant carcinoid syndrome to DAPH (Fig. 46), however, suggests further study of this question.



(A) (B)

Figure 46

Two small arteries from the lung of a patient with malignant carcinoid syndrome (other features of this case previously reported from this institution[101]). Note the intimal proliferation and the thin tunica media. *A* stained with Verhoeff-Van Gieson, X64; *B* stained with Goldner trichrome, X160.

On the basis of present evidence it is difficult to determine the cause of the generalized degenerative arteriopathy. An inherited or acquired (or both) defect of the tunica media seems likely, but its cause is unclear. Both the hypertension

and some of the arteriopathy in the lungs is related to recurring small emboli from right atrial thrombi due to sinus node infarction, and in turn some of the arteriopathy is likely due to hypertension. Most of the possible humoural substances which could cause pulmonary hypertension and arteriopathy seem less important in view of systemic arterial involvement with normal systemic arterial pressure; this does not exclude such substances as contributory factors.

What we see in the histopathology of the pulmonary arteries may only be the consequence of chance involvement of certain systemic arteries (those of the cardiac nodes, right atrium and adrenal glands, e.g.) and have relatively little to do with the basic process of a generalized degenerative arteriopathy. In other words it is possible we are being misled by the striking pulmonary arteriopathy which leads to one type of death from a generalized arterial disease, the other possible consequences of which we do not know. In particular we do not know what the natural history of the disease would be in cases where the arteries of the adrenal glands, right atrium and cardiac nodes may be spared.

Even without an adequtae explanation for the degenerative arteriopathy, its similarity to systemic atherosclerosis merits some discussion. This is particularly true since all three of these young women had considerable coronary arteriopathy indistinguishable morphologically from atherosclerosis, which is most unusual at their ages in their sex.

CONSIDERATIONS RELATIVE TO ATHEROSCLEROSIS AND ARTERIOSCLEROSIS

In their description of medial hypoplasia in "primary pulmonary hypertension" Gilmour and Evans[45] called attention to the applicability of their observations to systemic atherosclerosis. A better understanding of healing and repair in any arteriopathy may well shed light on the pathogenesis of arterial sclerosis.

From a study of atherosclerotic systemic arteries fixed while being distended by intraluminal fluid under pressure, Crawford and Levene[102-104] demonstrated that an atheroma in most cases did not protrude into the arterial lumen, the way we are accustomed to seeing it in fixed undistended arteries, but that the lumen retained quite a well-rounded shape and that the atheroma instead bulged into the media. The remaining tunica media beneath the atheroma was considerably thinned out. It was shown that this medial thinning could not be simple pressure atrophy due to the atheroma being pressed into previously normal tunica media, for it is mathematically impossible for the pressure on the media beneath a covering to be higher than that on normal media near by; in fact pressure on media beneath the atheroma is less.

If the tunica media is the site of focal degeneration, two consequences affect normal vascular hemodynamics (Fig. 47). First the focus of weakened tunica media may retain sufficient strength to prevent aneurysmal bulging, but would after atrophy present a cavity toward the lumen of the vessel. It may be expected that this cavity will become filled in order to restore a smooth contour to the inner surface

of the vessel. Whatever the exact filling process, whether by thrombus or by intimal proliferation, or both, the end result will be a "scar" histologically indistinguishable from an atheroma.

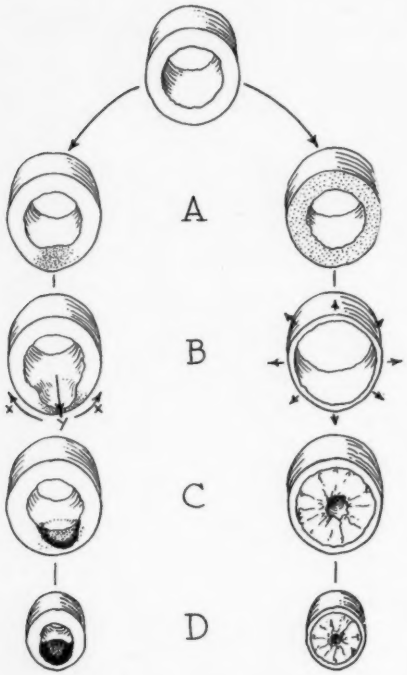


Figure 47
A schematic representation of the consequences of injury to a small focus (left vertical column) and to the entire wall (right vertical column) of an artery. Stippling at step A indicates the area of injury. In B the results of a loss of mural substance are stresses in the directions indicated by the arrows; with focal injury there are two stresses, one a direct centrifugal thrust favoring aneurysmal bulging (y), and the other a dividing force (x) since the intact smooth muscle encirclement of the artery has been disrupted. At the second step in the right column where there has been a symmetrical loss of substance of the entire arterial wall, the consequent weakening causes symmetrical centrifugal thrust and dilatation. In step C the healing of these two types of arterial damage is indicated; with focal damage the repair is a conventional atheroma, which fills the defect in the tunica media thus dampening the aneurysmal centrifugal thrust at that point as well as rejoining the intact remainder of the smooth muscle circle; with more generalized mural damage (right column) a symmetrical endothelial proliferation is the only means of reducing the arterial radius and restoring pressure (see text for discussion), since the damaged tunica media is unable to do this by spasm or contraction. Step D shows the arteries as they are viewed in an undistended postmortem state, the atheroma on the left bulging into the lumen. Both types of damage are seen in DAPH; the type in the right column is uncommon in any other disease.

The second consequence of a focus of weakened tunica media is an impairment of the efficiency of the unweakened remaining fibers of smooth muscle. For normal function the smooth muscle of the tunica media depends on the maintenance of an intact circle about the vessel[105,106]. When a segment of this circle is damaged, normal intravascular pressure will force apart the remainder of the circle and thereby increase the diameter of this segment of vessel. A healing of this defect in the tunica media would not only fill in the cavity on the inner surface of the vessel, thus discouraging the aneurysmal centrifugal thrust, but would also link together again the surviving smooth muscle fibers and restore their efficiency in maintenance of "active tension"[105] of the vessel wall. Most discrete atheromata admirably serve both these purposes.

After long-standing or repeated bouts of damage to the tunica media this simple manner of healing must be replaced by a disorderly process in which focal hemorrhage, neighboring degeneration, calcification, and superimposed thrombosis all contribute. By the time most systemic atherosclerosis becomes clinically manifest and by the time opportunity for histological study at necropsy occurs, the simple atheroma with its exquisitely efficient natural reparative process is no longer to be seen.

If the atheroma is initially an efficient reparative process, why do we then sometimes see arteries which are virtually occluded by simple atheroma? These may of course partly be artefacts seen in collapsed, undistended arteries. They may also be instances of a different process which is only morphologically similar, but they are probably the same process. One explanation may be the body has no efficient checking or suppressing mechanism once atheroma formation has begun. In different patients this may apply to all atheroma formation, or may differ from artery to artery at any one time or at different times. For example, patients with high levels of circulating fat moities may so fill any developing atheroma with these substances that it gorges and distends it to the point of occluding the arterial lumen.

Another explanation of why the body will construct an atheroma which eventually encroaches on the arterial lumen can be made, however. According to LaPlace's law, which has been shown applicable to normal vascular dynamics by Burton[105], pressure is directly proportional to tension and inversely proportional to radius. In application to blood vessels, $P = \frac{T}{r}$ is interpreted thus: P is the intravascular pressure, T is the tension produced by the vessel wall, and r is the mean radius from the center of a cross-section of the vessel to an average mid-point between endothelium and outer adventitia.

Accepting the applicability of LaPlace's law to the dynamics of blood vessels, it is axiomatic that any decrease in pressure due to failure of tension can only be compensated by a reduction of the vessel's radius. With a small focus of medial damage an atheroma may re-link the undamaged smooth muscle so it may restore normal mural tension, but with a large focus of medial damage this may not be possible and pressure can then be restored only by an intimal proliferative reduction in radius. The consideration of similar variations in these factors (P, T and r) as they relate to vascular pathophysiology is beyond the scope of the present discussion, but it is impressive that in all these the applicability of LaPlace's law remains valid.

In DAPH one of the hallmarks of the histopathology is the beautiful concentric reduction of the arterial lumina by radially oriented intimal proliferation (Figs. 1, 2, 17, 18, 32A). Less well recognized previously is the almost universal accompanying medial atrophy which sometimes involves the entire ring of tunica media in that plane. If the entire circumference of the tunica media at this point is weakened, a logical means of restoring local pressure, since the tunica media is unable to do this with constriction, is for the arterial radius to be reduced by concentric intimal proliferation.

Analogy of DAPH and systemic atherosclerosis, including similarities in their pathogenesis, is supported by the amount of coronary atherosclerosis present in the hearts of the three young women in this report. Significant coronary atherosclerosis is singularly uncommon in young women[107-109] except in the presence of diabetes, systemic hypertension or other vascular diseases, one of which may be DAPH. Though extensive coronary atherosclerosis has not been considered characteristic of "primary pulmonary hypertension," systemic arteries in this disease have previously seldom been studied with the care given to the pulmonary arteries.

There is no dearth of possible considerations on the etiology of the damage to the tunica media in any arteriopathy. Cystic medial degeneration can occur as a consequence of impairment of mural blood supply[110] and Schlichter[111] has produced such lesions by coagulating the adventitia of the aorta. The essence of what is being suggested, however, lies in considering the atheroma formation as secondary to such damage rather than responsible for it. This is not to say that intimal proliferation or hyperplasia, once begun, does not aggravate the medial damage, for it probably does. Whatever the initial cause for medial degeneration, thickening of the overlying intima (even for the salutary purpose of strengthening and repair) removes or certainly impairs one possible route of nourishment to at least the inner third of the tunica media.[112,113] Thus the artery while attempting to heal itself is partially defeating its own purpose.

SIMILARITIES OF DAPH TO COLLAGEN DISEASES, AND SOME IMPORTANT DIFFERENCES

Resemblance of some features of DAPH to collagen diseases is apparent, and it has been suggested that "primary pulmonary hypertension" is a variant form of this group of diseases[43]. Among these, notable similarities exist between DAPH and lupus erythematosus, Raynaud's disease, or scleroderma. In all these diseases a comparison of the typical case of DAPH to a typical case of lupus or scleroderma or Raynaud's disease allows one to distinguish with ease the striking clinical differences, which are too familiar to require repetition. It is more difficult to separate the atypical cases.

Histologically one of the significant differences is the lack of inflammatory cellular reaction to the degeneration in DAPH, whereas the vasculitis of the collagen diseases is usually associated with a periarterial cellular response.[114] Prevalence of the arterial lesions in various organs is of additional differentiating aid, the vascular pathology of the kidney and spleen in lupus being much more extensive than in DAPH; also in lupus the glomerular capillaries are a prominent site of involvement, whereas in DAPH these seem to be spared. This latter difference between the two diseases applies to other organs as well, lupus tending to spare medium size and larger arteries, whereas DAPH characteristically involves them. Finally, even though the lung and right atrium may be involved in collagen disease, this is an unusual occurrence, whereas these are the site of extensive pathology in DAPH.

Raynaud's phenomenon, a feature of Case 2, is now considered as etiologically non-specific.[115] It is commonly a feature of the clinical course in the collagen diseases, but is also commonly seen in association with many other diseases. Raynaud's disease has been reported to involve the pulmonary vessels,[116-118] but this is unusual. Since it has not previously been appreciated that systemic degenerative arteriopathy occurs in "primary pulmonary hypertension," it remains to be determined whether some of the reported cases of pulmonary involvement by Raynaud's disease or by certain collagen diseases may not actually be DAPH.

Undoubtedly this whole group of diseases is closely related, and a too rigid separation of them may be both difficult and undesirable. Shadings of difference exist, and abundant opportunity for misdiagnosis in either direction must occur.

MECHANISM OF SYNCOPE AND SUDDEN DEATH

Although this topic is the subject of a fuller development elsewhere,[34] a brief disucssion is appropriate to this report. In the 3 cases reported here the lesions involving the cardiac nodal arteries were of two types. The first was a hemorrhagic and cystic or granular degeneration of the tunica media of the sinus node artery with ultimate rupture into the sinus node (Fig. 7). The second type of lesion was a combination of both intimal and musculoelastic hyperplasia which produced luminal encroachment (Figs. 29, 30, 33, 35). That the second is a likely consequence of the first has already been discussed.

Concomitant with the development of these focal lesions in the nodal arteries in DAPH, the progressive disease in the pulmonary arteries has two effects relative to syncope and sudden death. The first of these is a compromise of the blood flow through the lungs which impairs filling of the left side of the heart and thereby cardiac output into the aorta and coronary arteries. The second, also a consequence of rising pulmonary vascular resistance, is a development of right ventricular hypertension and at least intermittently elevation of right atrial pressure with resistance to coronary sinus flow. The combination of decreased central aortic pressure and elevated right atrial pressure results in a marked reduction in effective coronary perfusion pressure.[119,120] This reduction in coronary artery flow may be expected to produce generalized myocardial hypoxia, but when it occurs in conjunction with lesions specifically compromising flow in the nodal arteries, an intense focal ischemia of the sinus node and AV node may be anticipated. In DAPH this focal ischemia is most likely the major determinant of the syncope and sudden death.

In addition to focal nodal ischemia, other factors *contribute* to the pathogenesis of syncope and sudden death. For example, distention of the right atrium is a powerful stimulus for a vagomimetic reflex which may depress function of the sinus node or AV node or both[121,122]; this influence would be expected to occur in secondary pulmonary hypertension of any etiology, suggesting that this factor alone is insufficient to produce syncope or sudden death except in occasional cases. Similarly ischemia of the nodes as part of a generalized myocardial ischemia, without the selective vascular involvement seen in DAPH, is apparently not sufficient of itself to produce clinical evidence of nodal dysfunction else it should be observed more frequently in any pulmonary hypertension. Analogous observations may be made regarding cerebral anoxia, pulmonary vascular reflexes, retrograde flow of right atrial hypoxemic venous blood into the sinus node and AV node, and other factors which can occur in any pulmonary hypertension. For further details regarding these mechanisms and a consideration of their applicability to the marked increase in hazard of cardiac catheterization in these patients, the reader is referred to the separate communication dealing specifically with them[34].

THERAPEUTIC CONSIDERATIONS

At present there is no satisfactory treatment for DAPH. On consideration of the various etiologic theories, appropriate therapeutic measures have been suggested and attempted with only modest and inconsistent success. The direct instillation of

acetylcholine or priscoline into the pulmonary artery has been shown to reduce pressure there[2,62,63], but this has been evanescent, the pressure immediately returning to pre-treatment levels when the drug was discontinued. Although this establishes that there is a component of spasm contributing to the pressure elevation in the disease, it is quite likely that the disease would progress even if the element of spasm were removed. One must even give some consideration to the possibility that without spasm (and the consequent high levels of pressure) perfusion of blood through some of the narrowed non-spastic arteries might fail altogether, with subsequent thrombosis and occlusion compounding the pulmonary perfusion problem. In addition, any of the pulmonary vasodilators reaching the systemic circulation may further reduce central aortic pressure and thereby worsen the already lowered effective coronary perfusion pressure.

On the contrary it is perhaps worth considering the employment of systemic vasopressor substances to raise central aortic pressure, especially during a syncopal crisis or similar acute deterioration in patients with DAPH. This would produce an elevation in effective coronary perfusion pressure and could be expected to improve the coronary circulation to the nodes and myocardium. Norepinephrin, which is a profound systemic pressor agent and weak pulmonary pressor agent would be preferrable to metaraminol in DAPH, for the latter affects both circulations equally. Against this possible benefit, however, must be weighed the potential risk of rupturing weakened arteries, as in the sinus node or lungs.

Because of the theory that "primary pulmonary hypertension" was due to multiple miliary embolization, use of anticoagulants has been suggested[12]. In consideration of the role of repeated small embolizations from a mural thrombus near the sinus node in DAPH, anticoagulants may be expected to be beneficial. This effect would only eliminate the thromboembolic component of the progressive disease in the pulmonary arteries, however, and would not necessarily alter the medial degeneration and compensatory intimal proliferation. If hemorrhagic cystic lesions of the sinus node artery and pulmonary arteries similar to those observed in Case 1 prove to be frequent in DAPH, the hazard they present in dissection and rupture under the influence of anticoagulants must be considered.

Because of the constant likelihood of sudden dysfunction of the sinus node and AV node in DAPH, the effect of digitalis and quinidine in this regard should be considered when they are chosen for therapy. This is not to imply they should be avoided in DAPH if conventional indications are present, but one should be aware of the potential results of depression of sinus node activity or AV node conduction.

Since the arteriopathy is devoid of inflammatory cellular response, there is no reason to anticipate benefit from adrenal cortical steroids. The possibility of an important role being played by adrenal medullary hormones deserves further investigation, however, and if they are important in the pulmonary hypertension and arteriopathy, certain measures are available to control this factor.

SUMMARY AND CONCLUSIONS

Based on observations in 3 cases, evidence is presented that the disease known

as "primary pulmonary hypertension" is a degenerative arteriopathy in which pulmonary hypertension is a devastating but secondary phenomenon. Because of the consistent finding of a pathologic process involving normotensive systemic arteries in these three patients, it is suggested that hypertension can not be the only cause of the degenerative arteriopathy.

Whether all cases presently classified as "primary pulmonary hypertension" prove to be DAPH or not, it seems apparent that DAPH is a distinct disease. In those cases of "primary pulmonary hypertension" in whom syncope or sudden death are features of the clinical course, a careful examination of the arteries to the two nodes of the heart is now important. It must be emphasized that this involvement of the arteries to the nodes is segmental, however, and anything less than a thorough study with sections made at most 2 millimeters apart may miss the diseased segments.

All three patients had adrenal arteriopathy, and in one of these it was extensive. Based on this and certain other considerations it is suggested that the role of the adrenal medullary hormones be further evaluated regarding the pathogenesis and evolution of certain features of DAPH.

In view of the demonstration of systemic arterial involvement in this disease which was previously generally considered to be almost exclusively pulmonary, a number of implications regarding other arteriopathies suggest themselves and have been discussed. Prominent among these is the pathogenesis of systemic atherosclerosis.

The expected benefits and hazards of certain forms of therapy are considered in light of these new observations.

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EXPERIMENTAL MULTIBAND TETRACYCLINE MEASUREMENT OF LAMELLAR OSTEOBLASTIC ACTIVITY*

H. M. Frost, M.D., H. Roth, A. R. Villanueva and S. Stanisavljevic, M.D.

ABSTRACT

Newly forming bone is labelled with tetracycline given in experimental animals' drinking water for three separate 24 hour periods, each 24 hour period being separated from its neighbor in time by 5 days. The first 5 day period is termed the control period, the second the experimental period. During the experimental period, an experimental drug or other experimental variable is introduced. After sacrifice fresh, wet, undecalcified cross sections of m/3 of femurs are made and stained with basic fuchsin by Frost's methods. By suitable microfluorescence methods concentric tetracycline labelled bands are seen in the bone sections, appearing much like the growth rings of trees. The distance between the first and second tetracycline band comprises the control band; this bone grew under control conditions. The distance between the second and third tetracycline band comprises the experimental band; this bone grew under experimental conditions. The mean value of the width of the experimental band as the percentage equivalent of the control band's width is determined, using a mcirometer eyepiece. This gives a qualitative figure of osteoblastic activity. A method of measuring osteoblastic activity quantitatively is described briefly.

INTRODUCTION

Previous reports described methods of measuring osteoblastic activity in man and the results of such measurements.[17,18,19,20,21] The results have necessitated revision of some of the current ideas of the local and systemic factors governing osteoblastic activity. Little is known for certain about these governing factors. Much misinformation and frank speculation on this subject has crept into medical literature and texts in spite of the sparse and conflicting nature of the factual foundation.[15]

If, as it seems to us, the factors governing osteoblastic activity must be studied anew,[16] then laboratory animals must be used and a method must be available which permits accurate and unequivocal measurement of new bone formation per unit time per unit volume of the animal's skeleton. This method must be sensitive, permit direct observation of the modality to be studied and must circumvent the variables and uncertainties afflicting other methods.

In the past, ingenious attempts have been made to measure osteoblastic activity, both in animals and in man. These attempts have revolved around three basic methods. Each of these methods unfortunately is afflicted by variables which cannot be controlled or measured. Each method also has associated technical features and problems which sharply circumscribe its use.

In brief, these basic methods are:

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Osteoblastic Activity

(A) BALANCE STUDIES. In this method the net bodily retention or loss of a substance such as calcium is measured. Osteoblastic and osteoclastic activities are merely inferred with this method, whether measurement is accomplished with or without the use of radioisotopes.[4] In the light of our persent knowledge this method is unacceptable when the duration of the study is on the order of a week or less.

The reason is that there are other means than osteoblastic and osteoclastic activities for the production of positive or negative balance over short time periods.[11]

(B) LABELLING NEWLY FORMED BONE. In this method a bone-seeking substance which can be identified in situ in undecalcified sections is used to identify new bone formation. This method of observing new bone formation is the oldest, it being first used with alizarin which is a component of madder root. In addition to alizarin, a few other dyes have been used and heavy metals such as lead and mercury have also been used, the latter precipitated in situ as their black sulfides.[28,31] Radioisotopes have been used extensively, coupled with autoradiography.[5,7]

These substances have had several drawbacks. They are toxic to the viscera when given in doses large enough for convenient working in subsequently prepared bone sections. There is also the possibility that they are toxic to the osteoblasts and alter their normal behavior.

Tetracycline bone labelling belongs to this method. As will be seen, when tetracycline bone labelling is coupled with proper sectioning and proper microscopic methodology, a powerful and versatile tool is available.

(C) BONE ACCRETION OF RADIOACTIVE BONE SEEKING SUBSTANCES. In this method the amount of a dose of radioactive substance which is later found deposited in a bone is taken as a measure of new bone formation.[3,8,30] The difficulty with this method is that inorganic bone-seekers usually also become adsorbed on or incorporated in the crystallites already present in the skeleton at the time of administration. The latter physicochemical process should, on theoretical grounds, be a varaible one from day to day and from animal to animal. This method therefore measures the effect of two variables simultaneously, without permitting a quantitative estimate of the relative importance of either.[11]

TETRACYCLINE BONE LABELLING

We owe a debt to Milch, Rall and Tobie for their publication of the fixation of the tetracycline antibiotics in mineralizing new bone.[26] Since their second report in 1958 we have learned the following about the physiology of this phenomenon.

During the time when the blood level of the tetracycline antibiotic is elevated most tissues are stained to varying degree by the drug. This includes most of the skeletal surfaces, such as the walls of the various vascular channels, the walls of osteocyte lacunae and of canaliculae, the crystallite surfaces in feathered bone, in new bone and the zone of demarcation of osteoid seams. (See Fig. 1)

Within 48 hours after administration of tetracyclines has ceased, all of the drug in the body has disappeared, as far as optical detection is concerned at least, with

Figure 1

Fresh, undecalified cross section adult human rib, basic fuchsin, about 300X. An Haversian system in the process of formation is seen in cross section. The Haversian canal lies in the center of the plate. The osteoid seam lies between the India Ink bars; the four bars at the bottom of the figure lie on the zone of demarcation referred to in the text. The zone of demarcation is the plane where initial mineralization of osteoid commences.

This is lamellar bone and lamellar osteoid. The formation of fibrous bone follows a slightly different route.

Tetracyclines deposited in the zone of demarcation are permanently fixed in situ. The direction of growth of new osteoid and of progressive mineralization of osteoid is centripetal, or towards the geometric center of the plate.

three exceptions. These three exceptions are tetracycline fixed (1) in the zone of demarcation of lamellar osteoid seams, (2) in newly mineralized fibrous bone, and (3) in newly mineralized hyaline cartilage. The tetracycline deposited in these three locations appears to be permanently fixed therein and disappears only when the tissue containing the drug is resorbed. The longest period of time between administration of tetracycline and subsequent demonstration in bone to our knowledge is 9 years. This was the time between the patients's receipt of the drug and his death; autopsy material revealed the bone labelled 9 years previously. Numerous examples exist in the Laboratory's files of labels 5 to 8 years old.[25] *All* new bone forming during the administration of a tetracycline is labelled by the drug.

Once fixed permanently in one of the three locations above, there is no apparent diffusion of the drug into the surrounding bone. There is some fading over the years, the intensity of the 9 year old label being roughly 0.2 that of freshly deposited tetracycline, doses being assumed equal on a milligram per kilo basis.

The zone of demarcation at which labelling of newly mineralizing lamellar osteoid is proceeding has a finite thickness, on the order of 4 microns in adult humans. It is thicker in the young than in the adult. This zone progresses in the direction of mineralization a certain amount each day. This amount may be termed the thickness of lamellar osteoid mineralized per day. In rabbits there is some evidence that the tetracycline retards either mineralization, elaboration of new lamellar osteoid, or both.[6,29] Evidence on this point is lacking in other animals and man at present. Nevertheless, this possibility makes it necessary in some manner to eliminate such an effect from any experimental use for measurements.

In properly prepared and mounted undecalcified sections, tetracycline may be seen in brightfield microscopy provided the dose of the drug was sufficient — over 25 mg/kilo of organism per day. Much lesser amounts may be detected by microfluorescence. Microfluerescence is necessary for accurate measurements. The orthdox but expensive ultraviolet microfluorescence illuminators are more than adequate.[26]

For limtied budgets, and for complete safety to the observer's eyes, a blue-light technique is recommended.[13] (See Fig. 2) Photomicrography may be done with either technique.

Under microfluorescence tetracycline deposited in bone appears a golden yellow, lying in and parallel to the lamellae. (See Fig. 3) Where cement lines cut across a moiety of tetracycline labelled bone, the demarcation is perfectly sharp within the resolving power limitation of the light microscope. The intensity of fluorescence is directly related to the amount of tetracycline administered per unit time. The width of the labelled bands is related to the rate of mineralization of new osteoid and to the total duration of administration of the drug but not to the dosage per unit time.

At any single instant of time, the thickness of the zone of mineralizing bone which is being permanently labelled with tetracycline is the same as the thickness of the zone of demarcation referred to previously and is on the order of 4 microns. The border between labelled and superimposed, newer, unlabelled bone is not sharp

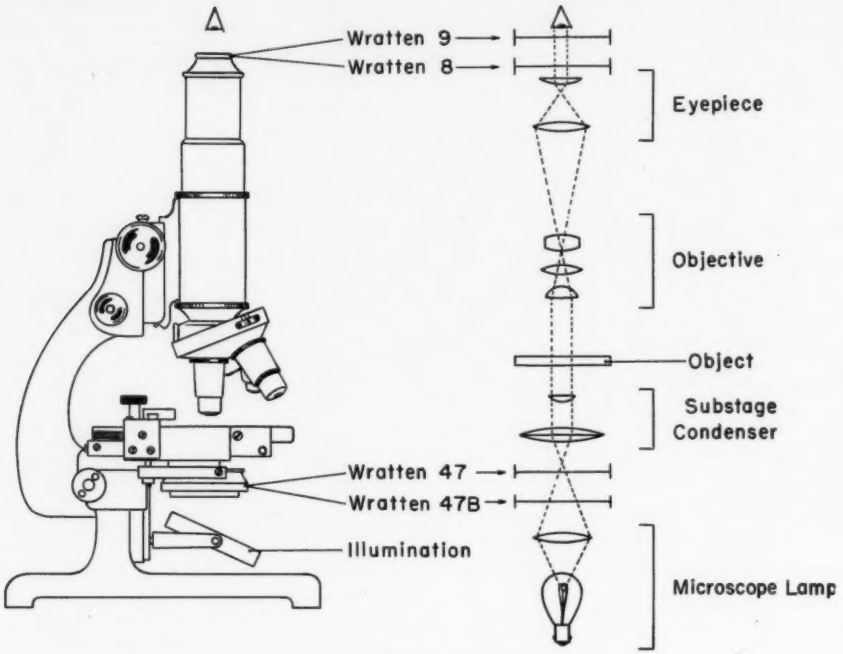

Figure 2

Diagram of a simple microfluorescence set up which is also safe to the observer's eye, even if improperly used. The light source may be a 35 mm slide projector of 200-300 volt-ampere (Watts) capacity. Kohler illumination should be used.[1,2,27] The blue-light filters (Wratten 47 and 47B) and the barrier filters (Wratten 8 and 9) are cemented between glass with nonfluorecsing resin such as H.S.R. These filters are obtainable as 2x2 inch gelatin sheets for 50 cents each. They are manufactured by Eastman Kodak.

Standard eyepieces, objectives and substage condenser are adequate.

All photomicrographs of tetracycline fluorescence in this article were taken with this setup. Standard mounting media may be used.

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but, at the resolution of light microscopy, is fuzzy. As a result, it is easier to determine the midpoint of a tetracycline band than it is to measure its thickness. This feature is compensated for in the present method of measuring osteoblastic activity.

No detectable tetracycline remains in sections after decalcification, regardless of the method by which this is achieved. Permanently fixed tetracycline in bone does not fade appreciably in the deep freeze, but does fade slowly in ethanol and formol. Surface tetracycline stains are progressively removed by aqueous, alcoholic and dioxane solutions and are best studied by fresh, frozen sections.[6,13,17,18,22,23,25,26]

MATERIALS

The essential materials and equipment will merely be listed here. Their use will become apparent as the Method is elaborated.

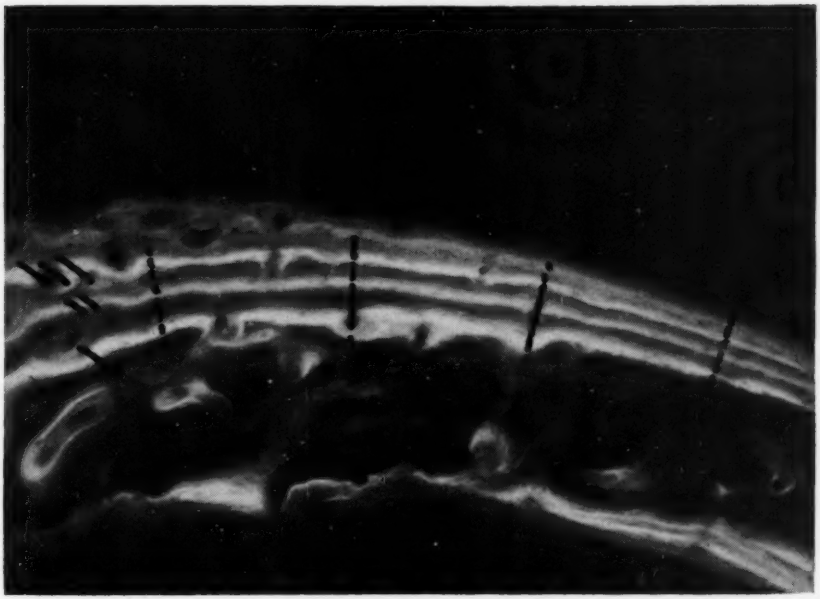

Figure 3A

Fresh, undecalified cross section middle third of femur of triply banded, 9 week old rat. 75X. The bright bands are tetracycline fluorescing with the set-up illustrated in Fig. 2. Periosteum is at the top, endosteum at the bottom. This was a control animal given tetracycline in drinking water for 3 separate 24 hour periods, each period separated by a 5 day interval during which no medication was received. Concentricity here is good and the resemblance to growth rings of a tree is plain.

The irregular areas lying in the cortex between the triple bands and the endosteum represent pseudo Haversian internal remodelling of the cortex. This section is 60 microns thick.

The fuzzy definition of the borderline between labelled and unlabelled bone may be appreciated in this figure, as well as in Figs. 4 and 5.

On the far left, the upper 3 diagonal ink marks identify the third labelled band referred to in the text (Methods, I); the next 2 diagonal marks identify the second label and the lower, single mark identifies the first label. The unlabelled bone between labels 1 and 2 is the control band. The unlabelled bone between labels 2 and 3 is the experimental band.

The dotted vertical lines illustrate where measurements of the thickness of the control and experimental bands would be made. A slight decrease in thickness of the experimental band is visible and is the result of the retardation of osteoblastic activity by maturation of the animal during the experiment. The animal was 6 weeks old at the beginning, 9 weeks old at the end of the experiment. This 3 week experimental period is 50% of the animal's initial age.

Optical: Medical microscope with mechanical stage having orthogonal movements. Standard dry objectives. A micrometer eyepiece[1,2,27] for qualitative work. For true quantitative work a calibrated micrometer eyepiece[1,2,27] and either a calibrated net micrometer eyepiece[1,2,27] or a calibrated Zeiss Integrating Eyepiece I[32] will be needed.

For orthodox microfluorescence, an ultraviolet illuminator, ultraviolet filter and barrier filters are needed; these are obtainable on order from most microscope manufacturers. For blue-light fluorescence microscopy, four Wratten filters and a 35 mm slide projector are necessary. (See Fig. 2)

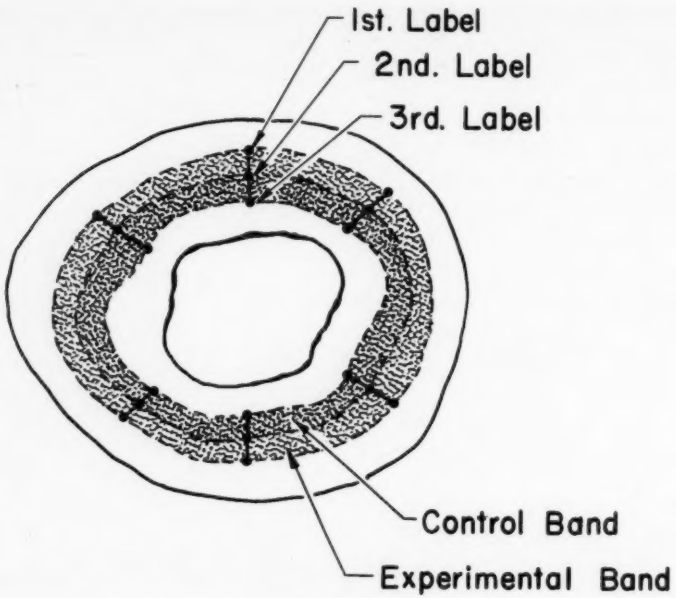

Figure 3B

Diagram of femoral cross section. The shaded bands represent the control (inner)and experimental bands (outer) referred to in the text. Such perfect concentricity is seen in the adolescent, and in adult rats; rats cease growing only upon dying.

Section preparation:[9,10] "Wet-or-Dry" or "Tufbak" carborundum surfaced sandpaper with waterpoof adhesive, grade 320 or 360, about 20 sheets. A flat surface such as plate glass, plexiglass or cookie tin. 1% or/and 0.3% basic fuchsin in 40% ethanol. H.S.R. or other suitable low-fluorescence microscopic mountant. Acid media may not be used. Assorted containers, slips, cover glasses. Source of running water.

Supplies: Achromycin, Terramycin, Aureomycin, or other brand-name of single or mixed tetracycline-based antibiotics. All of the currently avaliable tetracyclines or derivatives are suitable for the present work. The most water soluble ones will be the easiest to work with. A very fine toothed coping saw such as is used for cutting out children's puzzles is necessary to saw the slabs of bone which will subsequently be ground to sections. Rodent bones are unmanageably brittle with coarse-toothed saws.

METHODS

We first describe some details which have been published in detail elsewhere. Then the multiband tetracycline technique will be outlined as a series of steps.

Fresh, undecalcified, unfixed, ground sections are made by Frost's method[9] from the middle third of the diaphyses of standard bones of the experimental animals. The sections should be cut reasonably accurately perpendicular to the longitudinal axis

of the bone. The usual standard bones for this work are femur, tibia and humerus. Any long bone including rib is satisfactory from animals as large as or larger than adult cats.

The sections are stained in 1% basic fuchsin if from adult animals, and in 0.3% basic fuchsin if the animals were very young.[10] Unstained sections may be used but the contrast of the fuchsin is helpful in relating the morphology to the tetracycline labels. Staining time and solute should be as recommended.[10] Final section thickness should be about 50 microns.

After staining, sections should be hydrated in a tumblerful of water for 20 minutes and the surface stain then ground off both sides of the section. Sections are then dried in air and mounted in a nonfluorescent nonacidic microscopic mountant. This laboratory uses H.S.R., Harleco (Xylol). Non fluorescing media must be used with UV set-ups.

The experimental method follows as a series of steps. Where modification for other animals or purposes may be necessary, the reasons for the procedure are discussed so that modification may be done intelligently.

Figure 4

Fresh, undecalified cross section upper middle third of femur of triply banded 7 week old rat. 75X. The periosteum is above, the endosteum below. The rat was triple banded at 5 day intervals as described in the text, Methods, 1.

The 3 tetracycline bands are plain, and in places exist on the endosteal as well as periosteal surfaces. Concentricity is poor and there is considerable, irregularly oriented, internal remodelling of the cortex. Poor concentricity such as this is seen in younger animals and close to the metaphyses. For this reason sections are best obtained from the middle fifth of the bone. Note that the labelled bands are broad—the result of poor correlation with the long axis of the bone.

Measurements of a section such as this would have to be interpreted with caution. Measurements of the interior remodelling cannot be easily done due to variable obliquity of the new bone with respect to the longitudinal axis of the bone.

(1) *Expreimental Timing:* The present experimental animals are 6 week old rats. The total duration of the experiment is broken up into a number of periods of time, each period of time being reflected in the new bone produced in the femur and seen as growth-like rings on cross section. These rings are comparable to the growth rings seen in the stump of a tree. (See Figs. 3, 4, 5)

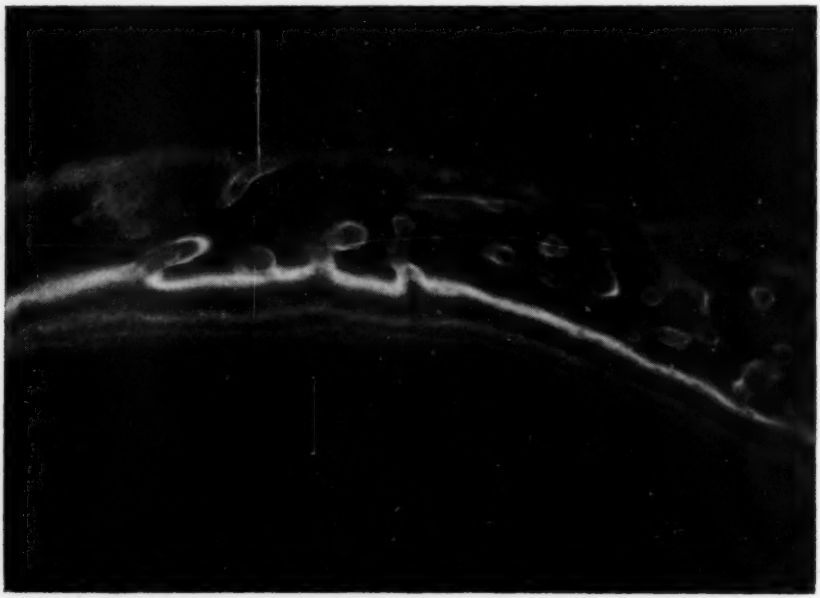

Figure 5

Fresh, undecalified cross section middle third of the femur of 8 week old rat. 75X. Microfluorescence. Triply banded with tetracycline. Given parenteral hydrocortisone during the experimental period, leading to retardation of lamellar endosteal osteoblastic activity. The value measured was 0.3 of normal, control band width (see text, Method, 7).

There has been no periosteal (top of figure) osteoblastic activity during the entire experiment. The bands illustrated are the result of endosteal osteoblastic acivity. The opposite quadrant of this section reveals periosteal banding, no endosteal banding, and a similar hydrocortisone effect.

(A) On day number 1 of the experimental period, the animals are given drinking water containing 1.0 to 1.5 gm/liter of the tetracycline antibiotic selected for use. Any new bone mineralized during this period, which should be exactly 24 hours long, will be labelled by the tetracycline. 6 week old rats drink about 20 cc/day and thus ingest about 200 mg/kilo. Plenty!

(B) From days 2-6 inclusive no tetracycline or drugs is administered. This is the control period and during this period the *control band* of new bone is being formed on top of the first labelled band. If injections of a drug are to be given during the later experimental period, dummy injections should be given during the control period because the physiological stress caused by the injections might affect bone growth (cortisone markedly retards lamellar bone formation).[21]

(C) On day 7 tetracycline is again given in the drinking water for exactly 24 hours. This produces a second tetracycline labelled band laid down on the preceding control and first labelled bands.

(D) On days 8-12 inclusive the experimental drug, surgical procedure or other change in the skeletal environment of the animals is introduced. This constitutes the experimental period and during this time the *experimental band* of bone is being deposited.

(E) On day 13 tetracycline is again given in the drinking water for exactly 24 hours. This produces the third tetracycline labelled band.

(F) The animal should not be sacrificed before day 16 of the experiment. The reason is that at least 3 days are necessary for more new bone to be formed to bury the third labelled band. A longer period of time before sacrifice is premissible as long as the delay is not sufficient to allow remodelling to destroy the three concentric bands. A fourth labelled band after a second control period and band may be indicated in some circumstances.

(2) *Design of the Experimental Timing:* The rate at which the experimental animal produces new bone governs the time intervals given in (1) above. In 6 month old rats and in 6 month old dogs the duration of each tetracycline label would be 2 days instead of one, and the duration of the control and experimental bands would be about 10 days, in some cases 15. Much longer periods of time than this will be unwise for most purposes because the total duration of the experiment is then apt to equal or exceed the time required to form the average Haversian system. This will lead to many partially labelled Haversian systems and this in turn leads to error in subsequent interpretation of measurements. This qualification does not apply if the animal does not form Haversian systems.

(3) *Need for Controls:* A unique feature of the present method is that the experimental animals serve as their own controls for a number af variables. For example, variation in level from which the section is cut, individual variation in reaction to food, environment, and like factors are eliminated because they exist in the same animal in the same place in the same bone for the duration of the experiment.

The effect of maturation on bone growth rate is not eliminated. In young animals such as 6 week old rats, the amount of maturation occurring between the control and experimental periods might affect the normal growth rate in those periods. For this reason, in very young animals in whom the total duration of the experiment is more than 10% of their age, controls must be run. The controls should be labelled in the same manner and for the same periods of time as the experimental animals but not given experimental treatment. Measurement, calculation and correction are described below in section (7). (See Fig. 3).

(4) *Sections:* Immediately after sacrifice undecalcified, cross sections are made which are cut accurately perpendicular to the long axis of the femur and through the middle third. These are stained and mounted as suggested.[9,10]

(5) *Observation of labelled Bands:* Under microfluorescence the tetracycline labelled femoral cross sections will appear about as in Fig. 3. Usually 3 concentric rings of tetracycline will be seen in those parts of the periosteal and endosteal surfaces where lamellar bone apposition was active. The perfection of concentricity, and the degree to which the three rings envelop the entier circumference of the bone, depend on a number of factors, among them being the age of the animal and the level from which the sections are cut. Concentricity is imperfect in the younger animals and imperfect in the neighborhood of strong muscle attachments, again more in the young than in the old. The illustrations purposely consider the more difficult situation to deal with: the young. (See Figs. 3, 4, 5)

(6) *Qualitative Measurement of Lamellar Osteoblastic Activity:* Imagine that the femoral cross section being measured is divided into pie-shaped fragments by radii placed as in Fig. 3. These radii should be separated by approximately equal angles. In adult animals or in adult Haversian systems the concentric bands will surround the entire circumference of the bone or Haversian system. In young animals they will usually surround only part of the circumference. Places where the labelled bone was deposited in such irregular fashion as illustrated in the cortical interior in Fig. 4 are ignored. There is justice in this deliberate omission because the bone forming in these areas is doing so under the aegis of different control and triggering mechanisms from that formed in the making of the concentric bands.

Along each of the radii diagrammed in Fig. 3, the width of the control and of the experimental band must be separately measured with the eyepiece micrometer. If the value for the control band be designated (c), and for the experimental band (e), then the ratio of the two is expressed as $\frac{e}{c}$, and designated as (oba) for osteoblastic activity. Slide rule accuracy is all that is required. Sufficient magnification should be used in these measurements so that the distance between the first and second labelled bands is more than 5 divisions of the eyepiece micrometer. The points of measurement are from the middle of one labelled band to the middle of the neighboring band.

The (oba) of all the measurements of all the sections of a single bone, such as the femur, are averaged and the mean determined. The figure obtained is designated as (OBA), and is the mean osteoblastic activity of the experimental band in terms of the control band as unity and normal. Multiplying (OBA) by 100 gives the osteoblastic activity of the experimental period as the percentage of normal. It is assumed that the control band width is 100% in this case.

In general about 50 or more separate determinations of (oba) should be averaged, obtained from at least 3 sections of each bone and from at least 4 animals, before an experimental effect is accepted as valid. When departures of experimental osteoblastic activity from normal lie in the range of 60%-140% of normal, more animals should be run before accepting an experimental effect as valid. Greater departures than this range may be provisionally accepted as valid with the numbers given.

(7) *Correction of (OBA) for Maturation Effect.* Assume that in a given experiment with cortisone this drug was found to retard lamellar osteoblastic activity

in the experimental band. The measured value was 0.3, or 30% of normal. (See Fig. 5). Assume further that litter-mates of this animal used as controls to determine the maturation effect on osteoblastic activity gave a value for the second tetracycline-free band (corresponding to the experimental band in the experimental animals) of 0.8, or 80% of normal. (See Fig. 3). The true, retarded osteoblastic activity produced by the cortisone in the experimental group of animals is thus $\frac{0.3}{0.8}$, or 0.37, or as percent 37%.

(8) *Differing types of Lamellar Osteoblastic Activity:* When measuring the effects of variables on lamellar osteoblastic activity, it must be realized that there are good reasons to anticipate differences in effects of some variables on Haversian, periosteal and endosteal lamellar osteoblastic activities.[25] For this reason, these three types of osteoblastic activity should be recorded separately rather than lumped together. Lumping them together is permissible only when the total, quantitative osteoblastic activity per unit time is being measured. This is a different phenomenon entirely and is the synthesis of the quantitative contributions of the three modalities named.

Note: The method as described so far provides a qualitative rather than quantitative measure of the various forms of lamellar osteoblastic activity referred to.

When Haversian osteoblastic activity is being measured, the values for a number of Haversian systems — preferably over 20 selected by a proper sampling technique — should be averaged. Single Haversian systems are measured as though they were cross sections of very small long bones.

(9) *Quantitative Osteoblastic Activity Theory:* A true, quantitative measurement of osteoblastic activity measures the volume of new bone formed per unit volume of the skeleton per unit time. This is desirable in certain types of work in progress in this and other laboratories. It is more difficult to do.[18]

The measurement may be achieved by measuring the total cross section area of sections made as recommended in Methods (4) and labelled as recommended in Methods (1). Then the total cross section area of the control and experimental bands should be measured. Because of the longitudinal grain of diaphyseal bone, the longitudinal grain of periosteal, endosteal and Haversian lamellar bone formation, and because 3 or more sections from any one bone will be measured, the results of such measurements may be reduced to unit volume measurements.[19,21,22]

Example: Assume the total cross section area of 3 sections from a particular rat femur is 11.6 mm^2. Assume that the total cross section area of the control band in these 3 sections is 2.1 mm^2. Then the control band, in terms of a single, average mm^2, is $\frac{2.1}{11.6}$ or 0.18. In other words, on the average for every mm^2 of cross section, there is 0.18 mm^2 of new bone formed in this animal. Because of the longitudinal grain and the fact that with several sections we have sampled the length of the diaphysis of the femur, we are justified in stating that for each mm^3 of diaphysis there is 0.18 mm^3 of new bone formed. This refers only to the middle third of the diaphysis. Metaphyseal formation must be separately measured by different and more complicated means.

In the above example the one thing still missing is the element of time. Assume that the control band was laid down over a 5 day period. Then in terms of new bone formed per mm^3 (the unit volume selected in this example) of diaphysis, per day, the figure 0.18 above is multiplied by $\frac{1}{5}$ to obtain .036 $mm^3/mm^3/day$. If, on the other hand, we wish to express the measurement in terms of a month, the value 0.18 must be multiplied by $\frac{30}{5} = 6$, and we obtain 1.08 $mm^3/mm^3/month$, or 1.08 mm^3 new bone formed per single mm^3 of skeletal tissue per month.

We recommend expressing results in a different manner than either of the above: the biological half-life of the bone. This means the period of time required for half the reference volume to be replaced with new bone, assuming that osteoblastic activity continued at the measured rate throughout this period and assuming that osteoclastic activity was equal. Neither of these two assumptions hinges on actual events and so neither need be verified by separate measurements. In the example above 0.18 mm^3 of bone was formed in 5 days; biological half-life is therefore $\frac{0.5}{0.18} \times 5$, or 2.78×5, or 14.9 days. The 0.5 figure is half of the unit volume.

The figure for biological half-life always indicates the time period in which half of any reference volume of bone (such as mm^3, cc^3, Liter, $inch^3$) will be formed at the rate measured. The biological half-life is thus readily convertible to other systems of measurement and, more important, permits immediate and valid comparison of measurements made at different times, in different animals and under different experimental conditions.

(10) *Quantitative Osteoblastic Activity: Practice:* There are many methods for obtaining the basic measurements required. Being microscopists we recommend the point counting planimetric procedure outlined by Hennig[24] and incorporated into the design of the Zeiss Integrating Eyepiece Micrometer I.[18,24,32] This procedure is explained in some detail in the references listed.

Briefly, the method works as follows: An eyepiece reticule containing a number of points (indicated by small crosses, or the intersections of the orthogonal lines of a net micrometer reticule) arbitrarily positioned in the reticule is superimposed on the image of an objective with N.A. between 0.3 and 0.5. Microfluorescence technique is essential. The reticule is calibrated with a stage micrometer[1,2,27,32] so that the exact, real area of the field being measured is known for the combination of objective and eyepiece used. (See Figs. 6, 7).

Fields are selected for measurement by a sampling technique outlined in other publications.[19,21] (See Fig. 8). Magnification is adapted to the thickness of the cortex and total cross section area of the section, being as low as the necessary resolution will permit in cross sections of human femurs, and as high as necessary in cross sections of small bones such as human rib or rat femur to fill the field with the thickness of the cortex.

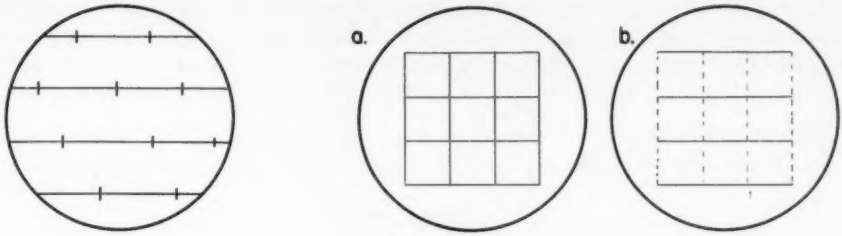

Figure 6

Diagram of reticule in Zeiss Integrating Eyepiece I (A) and of a typical net micrometer (B). The point of intersection of orthogonal lines is that referred to in the text, Methods, 10.

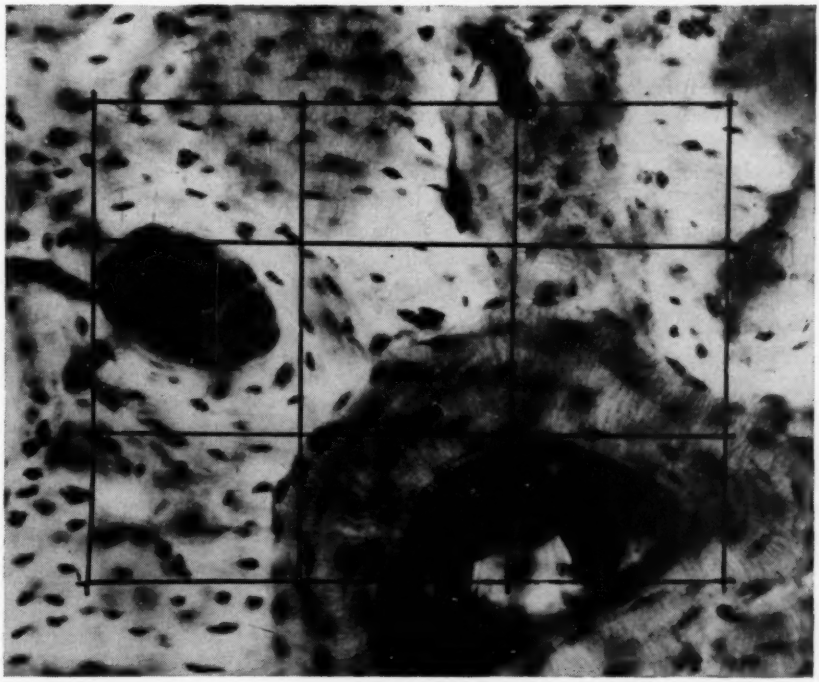

Figure 7

A typical net micrometer reticule superimposed on the image of a tibial cross section. There are 16 line intersections. Assume the vascular area of the section is desired, i.e., the total area composed of vascular spaces rather than bone. In the present case there are 2 "hits" on vascular channels and 14 hits on bone. If a large number of fields are measured in this fashion, and if the fields are selected by an adequate sampling procedure and if the total area of the field (which is larger than the area of the square) is known by calibration, and if the total number of fields and hits are recorded, the vascular area can be measured with good accuracy.

In measuring osteoblastic activity by the multiband tetracycline method, the same principle is used. Rotating the eyepiece without moving the section repositions the reticule points. Such repositioning may be counted as a separate field as long as all measured parts of the section are treated identically.

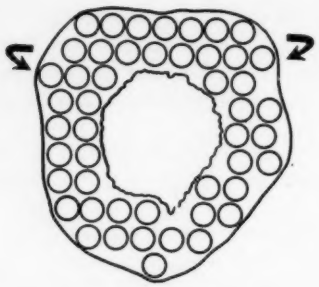

Figure 8

A method of sampling cross sections during quantitative measurements. The section is scanned in strips of one field wide. Each strip is counted in increments of one field diameter. The orthogonal motions of a mechanical stage are essential in such work. In large sections every other strip may be omitted.

Each field is measured by noting where the reticule points intersect with control (or experimental) band on one tally, where the reticule points intersect with other bone on a second tally. The intersections of reticule points with the microscopic image of the section are termed "hits". During the measurement of a single field the fine motion of the microscope may not be readjusted nor may the eyepiece be rotated. Separate measurements may be obtained by rotating the eyepiece an arbitrary amount between measurements on the same location of the section, recording each as a separate field on the third tally. This procedure is permissible only if all fields measured are all also treated in the same manner. Hits on the lumens of vascular spaces are not tabulated.

This procedure is similar to that diagrammed in Fig. 7.

At the conclusion of the measurements of, for example, 3 sections from the middle third of a single rat femur, all of the hits on control band (or experimental band) are added to all of the hits in the other bone. This total represents the total section area measured. Assume the total number of hits was 2000. Divide the total hits on the control band by this number, assuming there were 158 hits on the control band.

$$\frac{158}{2000} = 7.9\%,$$ the proportion of control band to total bone volume.

In terms of unit volume the control band is 0.079 mm^3/mm^3.

If the control band was laid down in 5 days, and we wish to state the osteoblastic activity in terms of the biological half-life of the middle third of the diaphysis, the calculation is as follows:

$$\frac{0.5 \times 5}{0.079} = 31.6$$ days biological half life, a real value determined on the rib of a 4 month old child. The figure 0.5 again is half the reference, unit volume.

The reader will note that in this procedure the real total area of the section is not measured. A sample of it is measured however, and this is the feature that

necessitates an adequate sampling procedure. By suitable choice of numbers of tallies periosteal, endosteal and Haversian lamellar bone formation may be measured simultaneously. Adequate precision with this method exists when the total hits on the smallest compartment being measured are in excess of 100.

Correction for maturation effect is done as described in section (7) above, after separate measurement of control animals has been done to obtain the necessary figures.

DISCUSSION

(1) The method described is suitable for measuring *lamellar* osteoblastic activity only.[12,14,15,16] It will not suffice for measuring the production of fibrous bone such as is produced in fracture callus, in certain bone neoplasms, or in the epiphyseal plate of growing animals. We have repeatedly referred to and emphatically again refer to this point. Too many investigators have mistakenly assumed that new bone is new bone and it is all alike. The result has been, as one example, the widespread misconception that protein anabolic hormones stimulate the formation of new lamellar bone.[15,16,18] It is true that administration of estrogen leads to increased amounts of fibrous bone in mouse and bird medullary cavities. Estrogen and its homologues do not stimulate the formation of lamellar bone in any animal known to us, including mice and birds. Since human postmenopausal osteoporosis (the disease most often treated with estrogen) is a disease of lamellar bone rather than fibrous bone, this observation is pertinent.

(2) The lamellar bone produced as endosteal bone apposition, the lamellar bone produced as circumferential lamellar bone apposition and the lamellar bone produced as Haversian systems are not necessarily governed by the same local and systemic factors, or not necessarily affected to the same degree by these factors.[25] This is a proper field for investigation with the technique described.

(3) It follows that endosteal and circumferential lamellar bone formation are best studied in growing animals; when growth has ceased these modes of new bone formation largely cease too. It also follows that the formation of Haversian systems, which is the mode of internal reconstruction of the cortex of most interest to us, is best studied in adolescent and adult animals; this mode of reconstruction is minor in the very young and in small rodents.

(4) While young rats, mice, rabbits and similar animals are very useful for experimentation because of their rapid growth and short life span, it should be emphasized that in many respects their skeletal physiology, anatomy and histology differ from that of man. It is therefore risky to draw parallels between such animals and man. It is quite permissible to use the laboratory animal as a testing ground, relying on human material to confirm the presence or absence of similar phenomena.

(5) The present method is of considerable value for the purpose of studying and discovering the various physical and chemical factors which affect the rate of osteoblastic activity in general. Note, however, that the measurements are on the middle third of one bone of an animal. Metaphyseal bone, bone of the axial skeleton and cacellous bone have been ignored for obvious technical reasons.

It will shortly become possible to normalize the osteoblastic activity of a standard bone to the osteoblastic activity of the entire skeleton, thanks to the collaboration of quantitative radioisotopic techniques with our quantitative histological techniques. This normalizing factor amounts to a calibration of the present method, as well as a calibration of methods previously published.[8,30] This calibration will considerably enhance the usefulness of the present method in both animal and human skeletal studies.

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BACTERIOLOGICAL AND CLINICAL STUDIES OF GENITO-URINARY TUBERCULOSIS

J. P. TRUANT, Ph.D* AND J. M. HODSON, M.D.**

Clincal urinary tract tuberculosis is generally described as taking one of two forms: 1) acute miliary and 2) chronic renal tuberculosis. In acute miliary tuberculosis the renal involvement is merely part of the generalized process. In this instance the infection usually involves both kidneys (95% of cases of Medlar *et al*[1].) is rapidly progressive and frequently fatal. The second type is often unilateral clinically and usually secondary to a focus of infection elsewhere in the body. Chronic renal tuberculosis may frequently be present in the absence of the original extra-renal source of the infection which has healed completely[2].

Since it is generally accepted that the tubercle bacilli reach the kidney by the hematogenous route[1], it would follow that the tubercle bacilli would lodge initially in the glomerular apparatus. Thus, the first lesion of renal tuberculosis is generally found in the cortical zone of the kidney. The infection may spread from the original tubercle to the medullary zone and subsequently to the lower urinary tract. Tuberculose cystitis of varying severity complicates the majority of cases and various investigators have described a high incidence of concomitant genital infection[3,4,5].

The symptoms of urinary tuberculosis are varied. If the kidney alone is involved there may be a complete absence of symptoms. The most common symptoms arise from lower urinary tract involvement. Progressive day time frequency and nocturia with bladder pain and dysuria are the most common manifestations of this complication. If renal symptoms are present they are generally secondary to ureteral or bladder involvement. Constitutional symptoms are not often present and when found may reflect extra urinary disease rather than genito-urinary involvement.

It is frequently said that renal tuberculosis is more common than has formerly been assumed. The reports of many investigators to this effect have been confirmed by the 5424 necropsy studies reported by Medlar *et al*[1]. Therefore, the bacteriologic diagnosis of genito-urinary tuberculosis is all the more important since the isolation and identification of *Mycobacterium tuberculosis* is frequently the only criteria for the recognition and/or confirmation of this disease. As a result, it is imperative that the most suitable bacteriologic methods should be adopted for the routine isolation and identification of tubercle bacilli from urine specimens.

In routine medical practice, urine specimens are submitted irregularly and often at long intervals, making it difficult to define the accuracy of the methods of culturing *M. tuberculosis*. Since extensive bacteriologic studies have shown that cultures positive for *M. tuberculosis* are not always found in all of the specimens, the intermittent nature of the discharge of these organisms into the urinary tract is emphasized. These facts stress the need for more frequent culturing of urine when tuberculosis of the genit-urinary tract is suspected. The bacteriological and clinical

*Department of Laboratories.
**Division of Urology.

data presented in this report will emphasize the necessity for culturing the urine frequently in cases of suspected urogenital tuberculosis.

MATERIALS AND METHODS

The majority of urine specimens were 24-hour specimens which were collected into a wide-mouth, 2-litre screw cap bottle without the use of any chemical preservative. Sedimentation of urine was improved by the addition of 2 or 3 ml. of 30 per cent acetic acid followed by the addition of 2 ml. of 5 per cent tannic acid (per litre) and the bottle allowed to stand for a few hours in the refrigerator. The sediment is subsequently removed and digested with an equal volume of tri-sodium phosphate (23 per cent Na_3PO_4. 12 H_2O)[6,11] at 37 degree C for an 18-hour period. The specimens are treated with IN HCL and adjusted to a Ph range of 6.5 — 6.9 to maintain optimal conditions.

The specimens are then centrifugalized at approximately 3000 RPM and the supernatant fluid decanted. Air-dried smears of the sediment are prepared on a slide which has been covered lightly with egg albumin. The air-dried smears are fixed by passing them through a low flame several times or by exposing them to a heating tray. The preparations are then stained by the Ziehl-Neelsen procedure[7]. Methylene blue is subsequently used as the counterstain.

Each specimen of sediment was inoculated into each of the three following media: 1) Petragnani 2) Lowenstein-Jensen 3) ATS-American Trudeau Society medium[9]. The cultures were incubated at 37 degree C until positive or for an eight-week period if negative. The specimens whose smears showed acid-fast bacilli were incubated for a 12-week period, but are presently being observed during a much longer time interval since it has been shown that some tubercle bacilli occasionally grow beyond the periods stated above.

Pathogenicity studies using guinea pigs were performed at the request of the clinician and both typical and atypical *Mycobacteria* were tested. Atypical acid-fast organisms have been studied more extensively during the past few years and have been identified by cultural, morphological and virulence tests with both guinea pigs and white mice. Differential methods which include tests of the biochemical activities of both *M. tuberculosis* var. *hominis* and the atypical acid-fast strains are in progress in our laboratory.

Susceptibility tests were performed because it is important from a therapeutic standpoint to know the degree of bacterial resistance. The technique for preparing suspensions of tubercle bacilli and procedures for the incorporation of chemotherapeutic agents are modifications of the methods described by Willis and Martin.[7]

RESULTS

A total of 707 patients submitted 880 specimens during a two-year period from 1959 through 1960. One-hundred and nineteen of these patients (17%) submitted 2 or more specimens (Table I). Among the group who submitted multiple specimens only 6 per cent of the patients were evaluated on the basis of three or more bacteriolo-

gical examinations. The importance of multiple specimens and their significance will be discussed later in this report.

Of the 707 patients, 21 or 2.96 per cent had urine specimens whose smears or cultures contained acid-fast bacilli (Table II and III). The data shown in Table II clearly demonstrates the value of examining both smears and cultures. It can be seen that 10 of 21 patients or 48 per cent had specimens which yielded a positive culture in the absence of observable tubercle bacilli on the smears. Four patients (19%) submitted specimens whose smears were positive and the cultures were negative. In seven patients (33%) both smear and culture were positive.

Table III is a brief clinical summary of patients who during the period of this survey demonstrated positive bacteriologic findings. The group was about equally divided as to sex and the ages ranged from 18 to 83 years. The highest incidence was found in those over fifty years of age. No patients in the pediatric age group were included in this study.

Of the seventeen patients with positive cultures, four gave a prior history of tuberculosis. Two of these had undergone nephrectomy, fourteen and sixteen years previous, for "unilateral" renal tuberculosis and another had been treated in the past for involvement of the hip. The fourth (WF) underwent enucleation of the left eye for tuberculosis at 2 years of age. This was some 17 years before the discovery of his urinary tract disease. None of these 4 patients showed evidence of active extra renal disease at the time of this study.

The most common presenting symptoms were frequency, nocturia and dysuria. Flank pain was present in two cases and one patient gave a history of gross hematuria. Ten patients were entirely asymptomatic and urologic investigation was prompted by the discovery of an abnormal urinalysis. Three of the four patients who presented with systemic complaints (fever, malaise, night sweats, etc.) demonstrated concomitant extra renal disease.

As is shown on Table III, all of the patients who had cultures which grew acid-fast bacilli showed an abnormal urinalysis. All but one had pyuria with or without microscopic hematuria. The patient (ES) without pyuria had microscopic hematuria alone. Eleven patients with positive cultures demonstrated albuminuria on routine urinalysis.

Table I

Number of Urine Specimens Obtained Per Patient.

No. of urine specimens	No. of patients
1	588
2	77
3	27
4	12
5	1
6	1
7	1
	Total 707*

*Note 119 of 707 patients submitted 2 or more specimens.

In seven of the seventeen patients with positive acid-fast cultures intravenous or retrograde pyelograms revealed no abnormality, and in only one were there changes pathognomonic for tuberculosis. Pyelographic changes in the remainder of the series could be ascribed with certainty to tuberculosis only in the light of the history, the clinical findings and the demonstration of *M. tuberculosis* in the urine.

Table II

Summary of Smears and Cultures Isolated from Urine Specimens of "Positive" Patients

PATIENT	LABORATORY NO.	RESULTS OF:	
		Smear	Culture
C. M.	10-14-2	−	+
	12-30-1	−	−
E. S.	11-2-5	−	+(ATYP.)*
	11-17-3	−	−
R. H.	1-9-14	−	+
	2-4-2	−	−
A. W.	9-15-2	+	−
	9-18-6	+	+
W. K.	2-24-60	−	−
	3-18-6	−	+
	3-24-4	−	−
E. C.	1-6-10	−	−
	1-7-7	+	+
	1-10-17	+	+
	1-11-7	+	+
W. O.	12-19-59	−	−
	12-22-3	−	+
M. B.	1-14-5	−	+
	3-29-9	−	−
J. G.	10-25-4	−	+
	1-28-8	−	−
J. H.	2-5-17	−	+
	4-22-3	−	−
	4-23-5	−	−
	4-25-11	−	−
	4-30-2	−	−
	5-2-5	−	−
	5-13-19	−	−
J. K.	7-19-3	−	+
	7-19-7	−	−

*ATyp.=A Typical.

Table II (Cont'd)

PATIENT	LABORATORY NO.	RESULTS OF:	
		Smear	Culture
H. S.	8-23-3	+	−
	8-28-1	+	−
	10-8-14	−	+
	10-17-60	−	−
	10-22-4	−	−
W. F.	1-3-8	+	+
	1-3-10	+	+
	1-4-8	+	+
	1-5-5	+	+
J. M.	2-23-60	−	−
	3-8-12	−	−
	5-15-6	−	+
	9-9-10	−	−
	12-6-5	−	−
C. M.	1-25-5	+	+
	3-8-11	−	−
	6-14-4	−	−
	6-23-8	−	−
	6-25-8	−	−
P. D.	1-21-59	+	+
	1-26-59	+	+
	7-27-7	+	+
	2-4-18	+	+
H. P. M.	2-5-7	+(ATyp)	+ Chrom*
	2-18-4	−	−
A. R.	2-28-59	+	−
	3-1-2	+	−
	3-14-5	+	−
E. H.	4-26-6	+	−
M. M.	5-13-7	+	−
	5-19-9	−	−
	5-25-1	−	−
M. Q.	9-24-2	+	−

*Chrom.=Chromogenic Strain (Photochrome)

Table III

Basteriological and Clinical Data on 21 Patients

Patients	Urinary Tract Symptoms	Urinary Sediment		Albuminuria	Pyelograms	Chest x-ray	History of Tuberculosis	Systemic Symptoms	Evidence of Other Organ Involvement	Smear	Culture	Age	Sex
		Leukocytes	Erythrocytes										
CM	Freq.[1] Dys.[2] Noct.[3]	+	+	+	Normal	Negative	Yes	—	—	O	+	46	F
ES	O	O	+	O	Normal	Negative	—	—	—	O	+	58	M
RH	O	+	O	O	Inflam.	Negative	—	—	—	O	+	18	M
AW	O	+	+	+	Hydro. Calc.	Active disease	—	Yes	Yes	+	+	61	M
WK	O (Prostatism)	+	+	+	Calc.	Negative	—	—	—	O	+	67	M
EC	Freq. Dys. Hemat.[4]	+	+	+	Non-Function (Unil.)	Negative	—	—	—	+	+	46	M
WO	Freq. Noct.	+	+	O	Normal	Negative	—	Yes	Yes	O	+	75	M
MB	Dys. Freq. Noct.	+	+	+	Calc. Inflam.	Negative	—	—	—	O	+	34	F
JG	Dys. Freq. Noct.	+	O	O	Normal	Negative	—	—	—	O	+	58	F
JH	O	+	O	+	Normal	Negative	Yes	—	—	O	+	38	M
JK	Dys. Freq. Flank, pain	+	O	+	Non-Function. (Bil.)	Active disease	—	Yes	Yes	O	+	71	F
HS	Dys. Freq. Noct.	+	O	O	Normal	Negative	—	Yes	—	+	+	62	F
WF	O	+	O	+	Inflam. Chg. (Unil.)	Negative	Yes	—	—	+	+	19	M
JM	O	+	O	O	Calc. Kidney	Negative	—	—	—	O	+	60	M
CM	O	+	+	+	Calculus	Not done	—	—	—	+	+	82	F
PD	Freq. Dys.	+	O	+	Hydro (Bilateral)	Negative	Yes	—	—	+	+		F
HPM	Freq. Dys. Flank, pain	+	+	+	Normal	Negative	—	—	—	+	+	58	M
AR	O	+	+	+	Normal	Negative	—	—	—	+	O	66	F
EH	Freq. Noct.	O	+	O	Normal	Negative	—	—	—	+	O	66	F
MM	O	O	O	O	Normal	Active disease	Yes	—	—	+	O	32	F
MQ	Freq. Dys. Noct.	+	O	O	Normal	Negative	—	—	—	+	O	57	F

1. Freq.=Frequency
2. Dys.=Dysuria
3. Noct.=Nocturia
4. Hydro.=Hydronephrosis
5. Hemat.=Hematuria
6. Calc.=Renal Calcification
7. Inflam.=Inflammatory changes
8. Unil.=Unilateral
9. Bil.=Bilateral

DISCUSSION

The literature on genito-urinary tuberculosis deals primarily with the clinical problems of diagnosis and treatment with little emphasis placed upon the bacteriological aspects. Delay in the diagnosis of renal tuberculosis results from a delay in establishing a bacteriologic diagnosis. One of the reasons for this is the acceptance of negative results, based on a single urine collection, as ruling out genito-urinary tuberculosis.

Since *M. tuberculosis* is difficult to culture from specimens even under favorable conditions it is especially necessary to accept the advice of the "Research Unit for Genito-Urinary Tuberculosis"[10] that the routine procedure should consist of at least three 24-hour urine specimens collected from each patient within a two-week period. This method, provides an excellent opportunity to conduct a reliable bacteriological study on each patient and will give a reasonably accurate bacteriologic status of the discharge of tubercle bacilli from urologic foci. If the patient is being treated with antituberculous drugs, urine cultures cannot be relied on entirely. For example, Kenney *et al.*[10] have shown that during isoniazid therapy sterile urine cultures do not necessarily reflect permanent arrest of renal tuberculosis. These investigators have shown that a waiting period of ten days after the cessation of therapy is necessary before cultures are reliable in determining the true bacteriologic status of patients being treated for urogenital tuberculosis.

Another common oversight in the evaluation of genito-urinary tuberculosis is the assumption that a negative smear for acid-fast bacilli is sufficient evidence to "rule out" the disease. It can be readily seen from our results (Table II and III) that 48 per cent of the patients' specimens had positive cultures in the presence of 'negative smear reports'. The data of other investigators[7] is in agreement with these results.

It has been generally accepted that 24-hour urine specimens should be used for the isolation of *M. tuberculosis*. This procedure is based on the supposition that tubercle bacilli may be present in such small numbers as to render isolation from small samples of urine very difficult. However, the collection of a 24-hour volume has certain inherent disadvantages:

(a) refrigeration of bulky 24-hour specimens during collection and handling is often cumbersome.

(b) contamination may present significant problems during the collection and processing.

(c) the collection may be impractical for ambulatory patients.

(d) these specimens necessitate an additional time period of 4-24 hours for sedimentation.

(e) not all the sediment can be processed and thus only part of bacillary population is cultured, thereby frequently defeating the primary purpose of the 24-hour collection.

Because of these disadvantages we have given some consideration to the reports of Kenney *et al.*[12] who have recently suggested the use of morning specimens instead of 24-hour urine collections on the basis of their experiences. A comparative study of these two methods by these investigators showed a greater percentage of recoveries of *M. tuberculosis* from morning urine specimens. Therefore we plan to evaluate the efficacy of this procedure in the very near future.

The symptoms of urinary tuberculosis are not specific and similar symptoms are present in many common urologic disorders. This fact along with the recent tendency to focus less attention on tuberculosis in general probably accounts for the frequent delay in diagnosis or its being missed altogether. These same considerations apply to the pyelographic changes and urine findings in genito-urinary tuberculosis. Although the results of pyelography or urinalysis may strongly suggest the presence of the disease the ultimate diagnosis rests on the isolation and identification of the organism in the urine. For this reason it is extremely important that the physician maintain a high index of suspicion in order to initiate proper diagnostic procedures and avoid serious delay in diagnosis.

Urinary tract tuberculosis should be suspected in any patient with recurrent or persistent urinary infection, prostatitis or epididymitis. A contracted bladder, ureteral stricture, or renal calcification should suggest its possibility as should any unexplained hematuria. Any patient in whom the disease is suspected should have a complete urologic evaluation. It is imperative that the physician be aware of the status of both the involved and uninvolved structures in the genito-urinary tract if future therapy is to succeed.

CONCLUSION

Eight-hundred and eighty urine specimens were obtained from 707 patients for microscopic and cultural examination. Twenty-one patients (2.96%) submitted one or more specimens which contained acid-fast bacilli. Of these, seven (33%) were positive for acid-fast bacilli by both smear and culture whereas the others were diagnosed by either microscopic or cultural methods. The report stresses the need for examining multiple urine specimens of patients who may have urinary tract tuberculosis.

The extreme variability of the clinical signs and symptoms of genito-urinary tuberculosis emphasizes the need for awareness on the part of the physician. This disease should be considered in any patient with recurrent and persistent urinary tract infection, prostatitis or epididymitis. A contracted bladder, ureteral stricture or renal calcification should suggest its possibility, as should unexplained hematuria.

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EXPERIMENTAL AND CLINICAL NEPHROSIS*

J. M. BANDERA, M.D., J. M. O'NEILL, M.D., AND GORDON MANSON, M.D.

DR. MANSON

In our last basic science seminar, Drs. Caldwell, Neher and Hamilton Smith dealt with the ultrastructure of the nephron which constituted a fitting introduction to our consideration this afternoon of the experimental and clinical aspects of nephrosis. Nephrosis is a systemic disease with disturbances in many areas of metabolism. In the broad sense of the word, there are many causes of the clinical syndrome we call nephrosis. These have been reviewed at length by Adams[1] in his excellent monograph. For the present seminar, however, we shall confine our discussion to consideration of a clinical entity characterized by proteinuria, hypoproteinemia and hyperlipemia. In the pediatric age group, this syndrome often is associated with one of four diseases: acute glomerulonephritis, it may occur during the course of chronic glomerulonephritis, it may be seen as a sequel to Schonlein-Henoch Purpura (historically more correctly referred to as Heberden's Purpura[2]) and it may occur as a familial disease. It is referred to variously as nephrosis, childhood nephrosis, or lipoid nephrosis. It is sometimes regarded as occurring in "pure" or "mixed" varieties.

Inasmuch as a major clinical manifestation of this disease consists of massive proteinuria and associated hypoproteinemia, we will ask Dr. O'Neill to deal with the disturbances seen in protein metabolism.

DR. O'NEILL

Nephrosis is a distinct clinical and biochemical entity. It is characterized by: edema, proteinuria, hypoproteinemia and hyperlipemia. Occasionally one sees hematuria, hypertension or azotemia. The pathogenesis of nephrosis is unknown, but it is commonly thought to be the result of an antibody reaction to unknown stimuli in a susceptible person.

While the causes of the disease in children are somewhat limited, in older age groups other causes are seen:

1. Intrinsic renal disease
    a. Membranous glomerulonephritis
    b. Mixed glomerulonephritis
2. Infection
    a. Syphilis
    b. Malaria
3. Poisons
    a. Heavy metals
    b. Organic solvents
    c. Drugs

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4. Hypersensitivity reactions
    a. Insect stings
    b. Serum sickness
    c. "Anaphylactoid" purpura
5. Mechanical
    a. Renal vein thrombosis
    b. Pericarditis
    c. Tricuspid insufficiency
6. Systemic disease
    a. Systemic lupus erythematosus
    b. Amyloidosis
    c. Multiple Myeloma
    d. Diabetes mellitus
7. Heredofamilial Nephrosis

Historically, "classical nephrosis" or membranous nephrosis, studied by light microscopy showed lipoid tubular degeneration, occasional increased cellularity of the glomerulus and splitting of the basement membrane. The lipoid granules in the tubules were considered the primary lesions of nephrosis. Actually, the lipoid granules of the tubules are the result of reabsorption of lipids that are the product of abnormal glomerular function rather than the primary defect in the disease. Elucidation of the primary lesion had to wait until the studies of the normal glomerulus were done by electron microscopy. Agreement on the histology of the glomerulus was no small struggle. Due to different staining techniques, artifacts and studies of various sections of the glomerulus, there was much controversy over normal glomerular histology. Now it is pretty well agreed that there are three layers in the normal glomerulus; endothelium, basement membrane and epithelium with podocytes. Farquhar, Vernier and Good[3] studied sections of the kidney from patients with various primary diseases having in common the presence of nephrosis. One lesion, other than the changes of the primary disease process, common in the glomeruli was the presence of degenerative changes in the podocytes. This lesion seems related quantitively to the severity of proteinuria. With the healing of the podocytes, proteinuria decreases. Hall[4] has proposed that the spaces or pores between the podocytes normally limit the molecular size of glomerular filtrate and that podocyte changes allowed larger protein particles to pass through and be lost in urine. Others have taken exception to this, postulating that it is not just a geometrical problem of pore size, but rather that there exists an enzymatic aspect to glomerular permeability and upon degeneration of the podocytes, there exists a biochemical defect allowing large protein particles to pass.

Before discussing the pathophysiology of nephrosis, a few comments as to age incidence of onset may be of interest. Acute glomerulonephritis has its highest incidence between four to ten years of age with a peak at about six to eight years.

Nephrosis

Nephrosis has its highest incidence from six months to six years with a peak of about 18 to 30 months. An episode of acute upper respiratory infection or acute tonsillitis is found to precede acute glomerulonephritis by two weeks in about 60 to 80 percent of cases, but in nephrosis this occurs only in 25 to 30 percent of cases. Some workers have suggested that infection is related to the onset of obvious edema in nephrotic patients only in that infection produces water retention in any child. However, in the yet unrecognized nephrotic child with inapparent edema, the added insult of infection may make the patient's nephrotic edema apparent for the first time. About 40 percent of nephrotics have a past history or family history of allergy, while this is much lower in the patients with acute glomerulonephritis.

Turning to the pathogenesis of nephrosis, two general views exist in regard to the primary defect; first, that nephrosis is a primary renal disease, more specifically a disorder of the epithelial podocytes. The second view is that nephrosis is a generalized metabolic disease with glomerular lesions being but a part of the process. The second view seemed a little vague until many excellent observations of a generalized capillary, metabolic, immunological, electrolyte, and hormonal disturbances of nephrosis are reviewed, then the theory of a systemic disease existing seems more logical. Without defending or trying to propagate any given viewpoint, let us try to evaluate each theory objectively.

Proteinuria is one of the most striking findings in nephrosis and may reach 20 to 60 grams per day. Proteinuria could be due to excretion of an abnormal protein, reduced tubular reabsorption of normally filtered proteins, or increased glomerular filtration. Immunological, chemical, and electrophoretic studies of urinary proteins rule out the first theory of abnormal portein production. The proteins excreted are normal serum albumin and globulins. Regarding the second theory, if 30 to 40 percent of serum proteins normally filtered by the glomerulus were not absorbed, one could account for all of the albuminuria. However, tubular reabsorption studies of albumin in normal and nephrotic patients is about equal. Microcannulation and Evan's Blue dye studies on experimental animals also confirmed the fact that protein absorption in normal and nephrotic animals was about equal. A third theory is that of increased glomerular permeability to serum protein. By micropuncture techniques, it has been shown that nephrotic kidneys have increased protein clearance. In human studies, infusion of nephrotic patients with dextran particles of molecular weights of from 10,000 to 150,000 with simultaneous fusion of tagged albumin and globulin, it has been shown that the dextrans of the molecular size of the various serum albumin and globulin molecules have been excreted in equal proportion to the concentration of albumin and globulin present. To add more support to this theory of increased glomerular permeability, one would expect the large molecules to pass less easily and the smaller molecules to pass more easily. This is borne out by the fact that the larger globulin molecules are found in lower concentration in the urine.

The source of the amount of proteins lost in the urine by the nephrotic patients is controversial in regard to the symptomalogy and total body nitrogen balance. Metabolic balance studies in nephrosis in the active stage showed markedly negative

nitrogen balance. However, such patients synthesize two to five times the usual amount of albumin and globulins; the total turnover of serum proteins is therefore greatly exaggerated. This has also been demonstrated with the use of albumin tagged with I_{-131} and tagged glycine molecules. The net result of proteinuria and greatly increased protein synthesis is reflected in serum by a low albumin, low alpha I gamma globulin, and low metal carrying globulins. Globulins of larger molecular size, the beta lipoglobulins and the alpha I lipoglobulins, due to an increased rate of synthesis and a decreased or at least not a very high rate of excretion, are actually increased in nephrosis, accounting for part of the hyperlipemia. Along with low beta lipoglobulin levels, one sees a low serum iron, low serum copper and low iron binding capacity. The protein-bound iodine is excreted in large amounts resulting in a low serum protein bound iodine value. However, normal I_{-131} uptake is present, excluding hypothyroidism as a factor in nephrosis. Gamma globulin synthesis is markedly increased and so is gamma globulin excretion; the net result is that the nephrotic usually has a very low gamma globulin. The role of protein intake on overall mortality is difficult to evaluate, but Blainey[5] and others have shown straight line correlations between nitrogen intake and storage. It is not possible to predict on the basis of patients' nitrogen balance whether or not recovery will occur.

DR. MANSON

No disturbance of metabolism in nephrosis is independent of other metabolic pathways. This is clearly illustrated by the observation that the infusion of 50 grams of salt-free human albumin is generally completely lost by the kidney within 24 to 72 hours from the time of infusion. Thus, albumin infusion is of more value to the physician's peace of mind than to the patient's nephrosis. An interesting observation in this regard is that such an infusion tends to produce a concomitant transitory decrease and subsequent rise in serum cholesterol level. Moreover, we note that in the nephrotic patient on steroid therapy, one generally must achieve a rise in serum albumin before the serum lipid level tends to fall. For a discussion of lipid metabolism and some of its inter-relationships with protein metabolism we shall hear Dr. Bandera.

DR. BANDERA

One of the metabolic disturbances found in nephrosis is hyperlipemia; the extent of the total lipids infrequently may reach a level of five grams. Although many of the metabolic inter-relationships of lipid and protein metabolism are known, an adequate explanation for the hyperlipemia has not yet been established.

In aqueous medium; that is, serum, lipids exist as joined colloidal lipid-protein complexes known as lipo-proteins. When the serum is analyzed electrophoretically, lipo-proteins are grouped into two fractions: (1) Alpha lipoproteins which are of lower molecular weight, high density, low lipid content lipo-proteins and (2) Beta lipoproteins which are high molecular weight, high lipid content lipo-proteins. Beta lipo-proteins normally transport about 70 percent of all plasma lipids in a normal fasting person. The fate of lipids in body fluids could be told in this simplified way: in the succus entericus the lipids exist as chylomicrons. Upon entering venous blood, by action of "clearing factor"[6], there is loss of triglycerides from the chylomicrons and the lipids are found as low density beta lipo-proteins. These in turn are transformed

to high density alpha or beta lipo-proteins. The "clearing factor" is thought to be a lipo-protein lipase. The following factors are thought to influence the activity or effectiveness of lipo-protein lipase; heparin, plasma proteins, and "tissue factor."

In trying to explain the hyperlipemia of the nephrotic serum, certain observations are useful. Although it is established that infusion of plasma protein will decrease the hyperlipemia of the nephrotic sera, the infusion of plasma protein has no effect on normal plasma systems. There is no diminution of lipemia. In experimental nephrosis, hyperlipemia precedes the diminution of serum proteins. Another pertinant observation is that in idiopathic hypoalbuminemia, serum lipids are normal but one can diminish serum lipids with infusion of albumin.

It is known that heparin will clear the lactescent nephrotic serum.[7] At least in certain experimental situations it has been established that the degradation of heparin is not increased. When heparin is injected into nephrotic rats and the rat's serum titrated with protamine sulfate, the prolongation of clotting time is similar in nephrotic serum as compared with normal control. Infusion of heparin to nephrotic rats with hyperlipemia does not diminish the extent of their hyperlipemia. These observations would exclude heparin, or lack of it, as the sole cause of hyperlipemia.

Despite low protein bound iodine and basal metabolic rate values in nephrotic patients, there is no evidence to suggest that the nephrotic patients are hypometabolic. The hyperlipemia of the nephrosis, although similar to that found in hypothyroidism cannot be influenced by the administration of thyroid extract. Marsh and Drabkin[8] have proposed a very interesting theory of hyperlipemia. They suggested that in nephrosis the body's main and most important effort must be to regenerate serum proteins lost in the urine. Since proteins are excluded from the metabolic functions they would normally participate in, such as gluconeogenesis, fats are called upon to carry the metabolic burden. Thus, the picture that one sees in nephrosis might represent mobilization of body lipid stores and the increased quantity in serum reflect an increased demand for them in the metabolic economy of the body. This situation could be rightly called "lipid diabetes."

The role of albumin in the hyperlipemia of nephrosis cannot be overlooked. Although it is thought that albumin serves as a 'carrying horse" for serum lipids, it is unlikely that its function is that simple. In this connection, the observation that the hyperlipemia persists despite the correction of serum albumin, as seen in clinical remission, supports the impression that there are other factors operating in the disturbances of lipid metabolism in the nephrotic patient. It is thus pertinent to remember that in acute glomerulonephritis, one sees hyperlipemia with normal serum proteins. The question of what constitutes the "clearing factor" cannot be answered as yet.

DR. MANSON

Disturbances in protein metabolism with their inevitable consequences in the area of lipoid metabolism also have a rather direct bearing on electrolyte and water metabolism. From the patient's point of view, edema may be his most distressing clinical manifestation. Edema formation, however, is not simply a matter of hypoproteinemia but also involves certain other factors. Dr. O'Neill will discuss these factors.

DR. O'NEILL

Edema is one of the major clinical findings of nephrosis and to parents this is one of the most distressing, ominous signs encountered. The clinician, however, must keep in mind that massive edema of nephrosis is but a reflection of many metabolic processes going on simultaneously to produce the net result — edema. Many factors operate to produce edema in any disease; in nephrosis, low protein, hormonal disturbances, water and electrolyte derangements contribute to the production of edema. Low protein is a result of increased protein catabolism and urinary loss. However, in other diseases with equally low serum protein concentrations, one does not see the massive edema of nephrosis; therefore, there must be other factors. Nephrotic patients have diminished plasma volume and occasionally this results in decreased renal blood flow with a shock-like picture which could account for edema on the basis of renal failure. However, this would not explain all cases of massive edema. Another possibility is the increased amount of antidiuretic hormone that has been found in nephrotic patients. This, however, is not a constant finding and could not explain all the cases of edema. In 1953 British investigators[9] observed that various extracts of urine from nephrotic patients showed marked sodium retaining activity and produced a potassium diuresis. These urinary extracts caused changes similar to those produced by crude adrenal extract; the active compound being later identified as aldosterone. This compound is present in increased quantities in nephrotic patients and is believed to play a large part in the often massive edema of nephrosis accompanied by extreme sodium and water retention and potassium excretion. The nephrotic patient can tolerate much larger excretory loads of potassium than the normal person but does not tolerate sodium and water as well. However, a major mistake made in treating nephrosis is to assume that limitation of sodium is essential to prevent edema. It is true that limiting sodium intake to some extent is necessary; however, a nephrotic patient seems to have a certain threshold level of edema which is the sum total of the hormonal and protein factors. When given more salt, the patient will increase his threshold for edema but beyond that point any excess salt is excreted. In treating nephrosis, acute limiting of sodium and the misery it causes these children, is certainly inexcusable on the basis of metabolic studies. Moreover significant sodium restriction is impossible, if good nutrition is to be provided. The mechanism for increased aldosterone production is probably on a basis of low plasma volume and as the sodium is diluted by the edema from other causes, osmo-receptors stimulate aldosterone production causing more sodium retention. Hydrocortisone will cause a rapid drop in aldosterone levels and the interesting observation is that in a spontaneous or induced remission, one sees a marked drop in aldosterone before diuresis occurs. One other possibility in nephrotic edema has been studied, that of generalized capillary permeability. Perfusion with Evans Blue tagged albumin substantiates the idea that, not only do glomerular capillaries have increased permeability, but the capillaries in other parts of the body as well. The actual quantitation of this phenomenon as to particle size has been very difficult, but there is clear indication that at least in part, the edema of nephrosis is due to generalized increased capillary permeability.

DR. BANDERA

As a clinical "rule of thumb," it is usually accepted that edema will appear when

the serum albumin in an adult falls to a level of 2.5 grams percent or less; or in a child under 10 years of age, the serum albumin level reaches 1.2 grams percent. In nephrotic patients, however, one often sees no discernable edema when the serum albumin is less than 1 gram percent. The analysis of the tissue specimens of edematous rats shows that the skin and subcutaneous tissues are the most avid acceptors of water. An excess of 1.9 grams of water per gram of tissue is retained in a nephrotic rat's skin. One gram of muscle tissue of a nephrotic rat has an excess of 0.5 grams of water as compared to the tissue of a normal rat. The analysis of electrolytes of a nephrotic rat's tissue shows that there is marked increase in sodium and chloride and decrease in potassium content when compared to the tissue of healthy rats.

DR. MANSON

So we see that a number of metabolic disturbances are involved in the clinical picture we know as nephrosis. These aberrations have been rather well worked out to date. Much work has been done and much remains to be done in elucidating the basic reason of why these disturbances occur. This brings us then to a consideration of experimental nephrosis and nephritis and the relationship of these experimental models to human disease. For this we shall call on Dr. Bandera.

DR. BANDERA

Experimentally, one can produce in animals a whole spectrum of renal diseases. These range from lesions clinically resembling human acute glomerulonephritis, to lesions resembling nephrosis and serum sickness. Many agents have been used: amino-nucleosides, nephrotoxic sera, intravenous saccharated ferrous oxide, trimethadione, just to mention a few. From these studies much knowledge has accumulated. Our correlation of anatomical lesions and clinical picture has become clearer; the biochemical aberrations and their sequence of appearance, inter-relationship and pathogenesis has been intensively investigated. Although the crowning of these experimental investigations would be definition of etiology (or etiologies) of human nephrosis, the fact that some renal lesions experimentally produced so closely resemble human nephrotic syndrome helps us to understand and manage the patients with the disease. It has been clearly established that, at least in the rats, nephrosis and nephritis can be diseases of hypersensitivity. Some investigators have followed a different path of investigation. They have been able to produce experimental nephrosis with subcutaneous injections of an amino-nucleoside, 6-dimethylamine-9 purine. This experimental approach has been studied by Bartlett at this hospital.[10 a-d] It has been shown by experiments using mitochondria prepared from pooled kidney tissue that there is a loss of mitochondrial activity in respect to both substrate oxidation, and concomitant phosphorylation, when succinate is used as a substrate. The following sequence of histologic changes has been established in experimentally produced nephrosis. Within one hour after administration of nephrotoxic sera to experimental animals, there occurs swelling of foot processes and increase in the amount of epithelial cytoplasm. In three hours swelling of epithelial cells takes place and in six hours there is coalescence of foot process. By 24 hours one finds proteinuria and the clinical picture of nephrosis follows soon after that. Amino-nucleoside induced nephrosis is somewhat different: the rat is the only susceptible animal. Although one is able to produce a full-blown picture of nephrosis with elevation of the BUN with this

agent, upon the discontinuation of amino-nucleoside induced nephrosis appears on the sixth day, edema and ascites appears on the tenth day, and uremia on the twelfth day. The main histologic change, which is somewhat different than in "hypersensitivity" nephrosis, is vacuolization of mitochondria within the distal tubules and breaking up of cristae.

Not only has production of experimental nephrosis been extensively investigated, but the modification and prevention of the disease has produced some interesting results. For instance, total body radiation, cortisone in a dose of 5 mg. per kilogram per day will produce significant suppression of disease. Marked or complete suppression can be attained by giving cortison in a dosage of 20 mg. per kilogram per day. Nitrogen mustards given in adequate doses will also prevent production of nephrosis by the use of nephrotoxic sera. It is significant that all these agents; radiation, steroids and nitrogen mustards are agents which we associate with inhibition of immunologic systems. In this connection it is worth pointing out that amino-nucleoside nephrosis is not influenced by pre-treatment with steroids.

The immunologic concept is frequently challenged by the fact that infants, who are usually considered "immunologically immature," will develop nephrosis. In this connection, it is worth pointing out that maternal antibodies against infants' kidneys have not been found.

Certain questions might be raised regarding the relationship of experimental nephrosis to the clinical nephrosis: a. Is there an abnormal nucleoside or nucleotide which functions as an antimetabolite and inhibits renal enzyme systems? b. Is there a "substance" which might be a by-product of an antigen antibody reaction which is responsible for the clinical picture of nephrosis? Although in azotemic patients abnormally high levels of nucleotides have been found, the relationship of those substances to nephrosis has not been established.

DR. MANSON

The possible role of the immune mechanism in the production of human nephrosis has been long apparent. Further substantiation of this concept has been emphasized by the discussion of experimental nephrosis by Dr. Bandera.

Clinically, it has been noted that atopic allergy is more common in families of nephrotic patients than in normal controls. Eosinophilia in these patients is not infrequent. It is equally clear, however, that atopic allergy together with a positive family history, hardly provides an adequate explanation of the pathogenesis of the disease. Other evidence of the role of the immune mechanism in this disease is to be found in the observation that complement is diminished in these patients. Serum complement also falls in experimental nephrosis. Furthermore, it has been demonstrated that this reduction in serum complement in both situations is not due to urinary loss in a manner analogous to serum iron, serum copper, protein-bound iodine, etc., as discussed previously. Localization of antibodies on glomerular components has been demonstrated in experimental disease but this observation has not been made in specimens from human nephrotic patients. However, in experimental disease these antibodies may remain attached to the glomerulus for months.

Antibodies to human kidney tissue have been studied but their presence in nephrotic patients has not been consistently demonstrated and the observation is open to considerable question. Boyden[11] has studied the sera of nephrotic patients by means of her tanned red blood cell technique. She reported that the sera of nephrotic patients produced agglutination of these specially treated red cells and showed that steroid therapy prevents this response. Other workers, however, have been unable to duplicate these results and so this observation remains open to some question.

Occasional isolated case reports of nephrosis in early infancy have been interpreted by some to imply that the immune mechanism is not as important as some have suggested inasmuch as the young infant is regarded as an immunologically immature individiual. Though this is in general true, it should be remembered that the immune response of young infants to test antigens is a complex problem because of the influence of passive maternal immunity to a wide variety of antigens. Furthermore, young infants may show a striking response to some antigens when maternal titers to these antigens are low.

In any event, similarities between human nephrosis and experimental immunologic disease are sufficient in number and nature to provide a rationale for the ablation of such a pathogenetic response by the use of steroid therapy. It might be pointed out in this regard that, utilizing the same rationale, nitrogen mustards have been used for the same purpose in the role of antireticuloendothelial agents.[12] We shall ask Dr. Bandera to discuss the present day therapeutic approaches to nephrosis.

DR. BANDERA

In planning a therapeutic regimen there are some considerations which influence the therapeutic success of a regimen. It has been found that in the case of nephrotic patients less than one year of age, the disease is usually not influenced by any therapeutic regimen. The duration of the disease has bearing on the responsiveness; the purity of clinical picture is rather important since, if one has complications such as positive tuberculin reaction or exposure to tuberculosis, prolonged disease with impaired renal function, or hypertension the approach is somewhat different.

Historically, one is impressed by the great mortality of nephrotic patients in the pre-antibiotic era. Barnes, et. al.,[13] in 1950 reported 42% of patients out of his series of 107 patients died when followed two or more years. Although antibiotic therapy lessened the mortality, the survival figure still left much to be desired.

Many articles have been written on efficacy of steroids or corticotropin and the clinical course of nephrosis. Most articles have reported favorable influence on the course of the disease and very little influence on the growth if the steroids are used intermittently.[14] Some investigators feel that corticotropin is the preparation to use; others have praised one steroid in preferance to another as far as the side effects, hypertension and the percentage of remissions is concerned. Certain serious side effects of steroid therapy have been seen in nephrotic patients. Consensus, however, is that steroids should be used intensively and not merely as diuretic agents to be stopped as soon as diuresis ensues, but to be continued until the biochemical abnormalities, hypoalbuminemia and albuminuria, disappear and there is a cessation of hyperlipemia.

Some investigators[15] report chlorothiazide will produce substantial diuresis. Threshold substances, such as Vitamin C, are occasionally used to induce diuresis. Although albumin will frequently produce temporary diuresis, it is worth pointing out that the pre-infusion level is usually reached within 24 to 72 hours and the course of the disease is not influenced by any such infusion even repeated daily. When after an adequate trial of steroids or corticotropin no response is obtained, frequently the nitrogen mustards are used with some success. In a resistant case one can use spirolactones, but our experience in this phase of treatment has been limited. Although steroids are thought to be rather specific, there are reports that nephrosis will return after remission of four years or more when the steroids are discontinued.[16]

One should not forget that supportive treatment such as adequate diet, reasonable isolation, and prevention of exposure to infections, especially viral, should be practiced when the patient is on high doses of steroids. Antibiotics, of course, do not influence the course of nephrosis but antibiotic treatment of bacterial infections has markedly reduced mortality from that major cause. Some also consider it good taste to put the patient on "prophylactic" antibiotics when long term steroid treatment is employed. This practice is open to serious question[17] and we no longer follow it in this clinic.

One should keep in mind that nephrosis is not the only thing that we are concerned with: the family, their attitude toward the manifestation of the disease, such as edema, profoundly influence our approach to it. Parents are often disturbed by the acneiform eruption or moon-face of the steroid treated patient. It is hard for them to accept that more than appearance is at stake when the patient is receiving steroids. Sympathy and an understanding attitude, combined with adequate explanation of what one is aiming at frequently is enough to change the parents' attitudes toward the disease, our management and the child's acceptance of necessary hospitalization and unpleasant venipuncture. In this regard, we must acknowledge the blessings of microchemical technics. The frequent criticism that we have no controls for the present regimen of steroid therapy is eloquently rebutted by the observation that our controls were all buried before the dawn of antibiotic therapy and may probably be used as an indication of the effectiveness of therapeutic antibiotics together with long term steroid therapy.[18]

DR. MANSON

Thank you, Dr. Bandera and Dr. O'Neill. Our time is drawing to a close and before it is up, I should like to emphasize a few points on which I think we all agree. The first is that nephrosis in childhood is a disease of unknown etiology whose prognosis has been vastly improved by two well established forms of therapy. It has been stated that with the use of antibiotics to treat bacterial infections, 40-60% of nephrotic children will ultimately recover. Most observers are of the opinion that with the use of adequate steroid therapy, as many as 70-80% of such patients will recover.[3] Secondly, if steroid therapy is to be used, and we feel strongly that it should be, these agents should be used in large enough doses for a long enough period of time. It has been established that adequate steroid therapy will reverse the abnormalities of the podocytes which are found on electron microscopy of needle biopsy specimens of the kidney from affected children and produce clinical remission. Thus we have objective evidence of the value of corticoid therapy in this difficult disease of childhood.

In our clinic, we have followed a prescribed program of treatment, in conjunction with the Children's Hospital of Michigan and the Department of Pediatrics and Communicable Diseases of the University Hospitals at Ann Arbor, which is part of a national study, designed to evaluate the long term results of therapy in this disease.[19] The fact that this study involves three institutions in the same corner of Michigan brings me to the third point on which we find agreement. Nephrosis is a disease which taxes the patience and confidence of parents and physician alike because of its natural history of remissions and exacerbations and its chronicity . . . the average case lasting two to three years, sometimes longer. The tendency is strong for parents of affected children to seek help at one institution and then another. This is clearly shown by comparing the rosters of nephrotic patients at one center with the other two. However, by a joint effort of the three hospitals involved, we have obtained a good follow-up on virtually all of the nephrotic children in this area for the past several years. The results obtained to date are presently under study and the project continues. The fact that nephrosis is such a trying disease to treat, brings into bold relief the importance of ancillary measures in the management of the patient. These include: orientation of the parents regarding nephrosis; prompt treatment of bacterial infections when they occur; minimizing exposure to infection, both bacterial and viral; education for the patient; maintenance of nutrition, especially insuring that the child gets as much protein food as possible; appreciation of the fact that steroid therapy is not without risk and careful evaluation of steroid induced complications when they occur, bearing in mind that nephrosis is a serious disease in which the side effects of steroid therapy must often be balanced against the implications of the disease.

Thank you gentlemen — we stand adjourned.

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ABSTRACTS OF RECENT PUBLICATIONS OF THE PROFESSIONAL STAFF OF THE HENRY FORD HOSPITAL AND THE EDSEL B. FORD INSTITUTE FOR MEDICAL RESEARCH

INTERACTIONS OF HYDROCORTISONE AND THYROTROPIN ON THYROID SECRETION OF INTACT DOGS. N. B. Ackerman, R. W. Smith, Jr. and J. M. Miller. Metabolism 10:27, 1961.

The effects of cortisol on the release of hormones from the thyroid gland of dogs were studied by serial measurements of PBI^{131} levels in blood from the thyroid vein. Infusion of 100 to 150 mg. of cortisol for one to three hours produced no appreciable change in levels of thyroid vein PBI^{131}. Three hours of TSH infusion (0.2 to 20 units) rapidly accelerated PBI^{131} release during and after the infusion. When cortisol was administered simultaneously with TSH, the usual accelerated release of thyroid hormone was inhibited. This depressive action of cortisol was brief and disappeared as plasma corticoid levels decreased after the cortisol infusion was stopped. When TSH administration was maintained after stopping the cortisol infusion, a secondary increase of thyroid vein PBI^{131} resulted. In animals with renal arteries ligated bilaterally, cortisol produced no comparable modification of TSH effect on thyroid secretion. These findings suggest that at least one possible effect of cortisol on thyroid function may be to increase TSH inactivation or sequestration by the kidneys.

LEG ULCERS IN RHEUMATOID ARTHRITIS: USE OF PRESSURE-GRADIENT THERAPY, HIGH-PROTEIN DIET AND OTHER MEASURES PRODUCES SUCCESSFUL RESULTS. J. Beninson and D. C. Ensign. J.A.M.A. 175:437, 1961.

Our study of 9 patients with leg ulcerations complicating rheumatoid arthritis showed that pressure-gradient therapy was successful. The therapy involved "pressure-sandwich" dressings with use of a pressure-gradient technique to heal the ulcer, and which, if correctly applied, eliminated the associated intractable pain. A diet high in protein was stressed which shortened the healing time, and the healed state of the ulcer was maintained by using custom-tailored pressure-gradient supports, measurements for which were determined by the physician. When necessary, standard metatarsal bars attached to the outside of the shoe were prescribed to correct pes planus deformity. Moderate walking exercise in these supports was advised to control edema. A morphologic classification of the ulceration for prognostic purposes was developed.

SIX YEARS OF PRESSURE-GRADIENT THERAPY; A GENERAL SURVEY. J. Beninson. Angiology 12:38, 1961.

The various usage to which pressure-gradient therapy has been put to date have been mentioned and anticipated usages suggested. Doubtlessly, other applications are inevitable. Perhaps they will one day be employed by man in space flight. Obviously much research will be necessary to elucidate the nature of the changes induced by pressure-gradient therapy in all the tissues involved. We are indebted to the now

deceased Conrad Jobst for his contributions to the treatment of people afflicted with leg ulcers and edematous limbs.

MODIFIED NECK DISSECTION FOR THYROID CARCINOMA. M. A. BLOCK AND J. M. MILLER. Am. J. Surg. 101:349, 1961.

The presence of palpable cervical lymphadenopathy in patients with carcinoma of the thyroid nearly always indicates the presence of metastases to the nodes. A radical neck dissection is indicated in the surgical treatment of most of these patients who are otherwise operable (primary lesion can be removed). In our experience at least one-third of patients with carcinoma of the thyroid but without palpable cervical lymphadenopathy do have metastases to the cervical nodes. Whether or not cervical node dissections should be used for these patients is, therefore, not decisively established. However, a modified neck dissection is considered justified in the surgical treatment of many of this group of patients and better results are suggested if it is done. A modified neck dissection has been defined as a dissection which is identical to the classical radical neck dissection except for the preservation of the sternocleidomastoid muscle and the submaxillary gland area. This includes removal of the nodes adjacent to the thyroid and pre-supposes complete removal of the primary lesion.

PERORAL JEJUNAL BIOPSY IN A PATIENT WITH DIABETIC DIARRHEA: A CASE REPORT. T. BRIDWELL AND F. W. WHITEHOUSE. Diabetes 10:58, 1961.

The "diarrhea of diabetes" was first described in 1936 by Bargen et. al. It is characterized by frequent, watery stools usually nocturnal in occurrence. Intermittency of symptoms and fecal incontinence further delineate this form of diarrhea. It is generally associated with diabetes of long duration which has been inadequately controlled. An associated peripheral neuropathy is common, and, indeed, it is accepted that diabetic diarrhea is merely another manifestation of the neuropathy of diabetes. Diabetic diarrhea usually is classified along with the neurogenic bladder, impotency, and postural hypotension as a form of "autonomic neuropathy." The jejunal mucosal pattern in a patient with diabetic diarrhea is reported herein.

MYELOMATOSIS WITH XANTHOMATOSIS. B. FRAME, M. R. PACHTER AND R. K. NIXON. Ann. Int. Med. 54:134, 1961.

A case is presented in which myelomatosis was associated with hyperlipidemia and skin xanthomata. Discussion of the deranged lipid and protein fractions in the patient's serum is related to previously recorded serum lipid changes in multiple myeloma. It is possible that, in certain cases of myeloma, the plasma cells may directly produce abnormal lipid fractions, or that a sufficiently abnormal lipid-binding protein is secreted to account for the serum lipid aberrations in the disease.

***STUDY ON THE DETERMINATION OF HEAVY WATER IN PLASMA OR URINE.** O. H. GAEBLER AND H. C. CHOITZ. Clin. Chem. 6:549, 1960.

A technic has been described for determining the excess of deuterium oxide in plasma or urine, by directly measuring 19:18 ratios in vapor from these fluids with a mass spectrometer of suitable design. Although the 19:18 ratio for water varied with sample size and other experimental conditions, the increment in ratio per unit concentration of added D_2O remained quite uniform. A virtually linear relationship between the increment in ratio and the concentration of added D_2O also facilitated checking the calibration. The time required for flushing out deuterium when a Pyrex inlet system was used could be minimized by carrying out both the calibrations.

AIR EMBOLISM. T. N. JAMES. Am. Heart J. 61:423, 1961.

The entrance of air into the circulatory system may or may not be fatal, depending among other things on the volume of air injected and the rate of injection. It has been estimated that at the same rate of injection the minimal lethal volume of air entering the right side of the heart is seventy times that entering the left side. Sudden death after the entrance of air into the left side of the heart is due to occlusion of the coronary arteries; death also follows occlusion of the cerebral arteries by the same volume of air, but in dogs this occurs only after many hours. So long as the heart can maintain normal arterial perfusion pressure, air will be pushed through the capillaries into the venous circulation. That air under normal systemic perfusion pressure will readily pass a capillary barrier is not generally appreciated.

ANATOMY OF THE HEART: NORMAL AND PATHOLOGIC, AS RELATED TO CARDIAC FUNCTION. T. N. JAMES. In, American College of Chest Physicians: Clinical Cardiopulmonary Physiology, ed. by Burgess L. Gordon. Ed. 2, New York, Grune & Stratton, 1960, p. 8.

Although a knowledge of anatomy of the heart is a prerequisite in the considerations of the pathology and physiology, such information has assumed perhaps greatest significance in cardiac surgery. Thus, with the birth of cardiac surgery there has been a resurgence of interest in the meticulous descriptions of Vesalius, Vieussens, Spalteholz and others. This rediscovery of old facts will be of benefit to all of us. In the present review of the normal and pathologic anatomy of the heart, emphasis is directed to features that are of particular clinical significance. For example, the section on coronary artery anatomy is expanded because of the clinical interest in coronary disease; the more detailed considerations are omitted due to availability in standard references on gross anatomy. It should be mentioned, however, that certain features which currently seem most important may assume lesser roles in the future, owing to advances in medical knowledge. This scholarly article describes pericardium, myocardium, endocardium, cardiac valves, conduction centers and pathways, innervation, coronary arteries, veins of the heart, and lymphatic system of the heart, each in normalcy and disease.

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CORONARY ANATOMY FOR THE PRACTICING INTERNIST. T. N. JAMES. Minnesota Med. 43:847, 1960.

If knowledge of the exact location of a coronary occlusion is not important, then an enormous amount of present effort for the production roentgenograms of the coronary arteries is being misdirected. It is of course important, but not all the reasons are fully appreciated. An acute coronary occlusion is not, like Gertrude Stein's rose, just an acute coronary occlusion. It behaves differently depending on where the subseqent myocardial infarction occurs. Consideration of just a few simple points of human coronary anatomy can help explain this difference in behavior. Although many myocardial infarctions (perhaps the majority) are streaky or lamellar or diffuse, enough of them involve specific localized areas to warrant their consideration. Of these discrete infarcts, there are three groups, each of which is due to occlusion of one of the three main coronary arteries: Anteroseptal infarcts are due to occlusion of the left anterior descending coronary artery. High lateral infarcts are usually due to occlusion of the left circumflex artery. Posterior infarcts are due to right coronary artery occlusion. Mixed infarcts are better understood if the three basic infarcts above are remembered. *Anteroseptal infarcts* are the commonest of these three. The primary electrocardiographic changes are in leads I, aVL, and V2 to V4. Other factors being equal, more proximal occlusions of the left anterior descending artery produce larger infarcts. *Laterial infarcts* involve the obtuse margin of the left ventricle, usually more toward the base than apex of the heart. Electrocardiographically the primary changes are in leads I, aVL, and HV3 to HV5 (high leads); the conventional precordial V leads are conspicuously normal or show only the edge of the infarct. *Posterior infarcts* are the most interesting from the standpoint of coronary anatomy. Since 90 per cent of humans supply the crux of the heart (where most "pure" posterior infarcts occur) via the right coronary artery, it follows that 90 per cent of these infarcts will be due to right coronary occlusion.

PHYSIOLOGY AND MORPHOLOGY OF BLOOD COAGULATION IN POLYCYTHEMIA VERA. T. N. JAMES, S. A. JOHNSON AND R. W. MONTO. J. Appl. Physiol. 15:1049, 1960.

The observation that red cells fall out of the clotted blood in polycythemia vera has been documented previously. This phenomenon appears in all patients with initial thrombocytosis but continues to occur even when the red cell mass and platelet numbers come under control. The only known coagulation defect is a pronounced reduction of platelet factor$_3$ in the platelets. In this investigation, platelet-plasma clots from normal individuals and polycythemia vera patients were sectioned and stained at various intervals during coagulation and clot retraction. Great disorganization of the platelet-fibrin network was observed in the polycythemia vera clots when compared with the normal clots, and it was apparent that the red cells could easily slip through the openings in the network.

PRIMARY HYPERPARATHYROIDISM: A DIAGNOSTIC CHALLENGE. W. C. MIEHER, JR., V. THIBAUDEAU AND B. FRAME. Arch .Int. Med. 107:361, 1961.

A series of 21 proven cases of primary hyperparathyroidism diagnosed between

1931 and 1959 is reported. From an analysis of the cases reported and a review of the recent literature, the various clinical syndromes presented by hyperparathyroidism are discussed and the laboratory and x-ray aids to diagnosis are reviewed. The authors are of the opinion that the key to the more frequent diagnosis of hyperparathyroidism involves the screening by serum calcium and phosphorus determinations of patients with the following conditions: (1) Urinary calculi or renal calcinosis. (2) Recurrent or chronic urinary tract infection; unexplained hermaturia and azotemia. (3) Demineralizing or osteolytic skeletal disease. (4) Symptoms of peptic ulcer, with or without x-ray evidence of ulcer. (4) Pancreatitis and/or x-ray evidence of pancreatic calcinosis. (6) Recurrent or persistent vomiting of unexplained cause. (7) Sustained hypertension especially with renal damage. (8) Unexplained behavioral changes in previously well-adjusted persons. (9) Unexplained polydipsia and polyuria. (10) Patients previously operated upon for parathyroid adenoma (should probably be checked yearly). (11) Patients with symptoms of islet-cell or pituitary adenomas and (12) Family history of hyperparathyroidism.

*ASSAY OF THE DPNH DEHYDROGENASE OF THE RESPIRATORY CHAIN IN PARTICULATE AND SOLUBLE PREPARATIONS. S. MINAKAMI, R. L. RINGLER AND T. P. SINGER. Biochem. & Biophys. Res. Comm. 3:423, 1960.

This paper describes the assay conditions developed for following the activity of the DPNH dehydrogenase of the respiratory chain during purification. An assay has been elaborated which measures the full activity of the enzyme in organized subcellular systems as well as in the soluble, purified state. By means of this assay, as described in the companion paper, it has been possible to extract in soluble form for the first time the enzyme in question, using heart mitochondria as a source. Judicious use of this assay enables one to localize exactly the site of action of numerous metabolic inhibitors of interest, such as barbiturates.

MYXEDEMA NEUROPATHY AND MYOPATHY: A CLINICAL AND PATHOLOGIC STUDY. S. N. NICKEL, B. FRAME, J. BEBIN, W. W. TOURTELLOTTE, J. A. PARKER AND B. R. HUGHES. Neurology 11:125, 1961.

Manifestations suggesting neurologic and muscular impairment were sought in 25 patients with primary myxedema. Objective sensory and motor dysfunctions with decreased peripheral sensation and proximal muscle weakness were significant findings in this study. The pathologic alterations in nerve and muscle consisted of mucinous deposits in the endo- and perineurium as well as in the endo- and perimysium. The myelin sheaths and axis cylinders were altered. Both structure and staining changes were present in muscle fibers. The serum studies revealed a normal total protein concentration; however, separation of the various protein fractions revealed significantly decreased albumin and increased beta and gamma globulin. The increased gamma globulin was confirmed by an immunochemical technic. The total serum cholesterol and total serum lipoproteins were elevated significantly. Furthermore, the alpha-2 globulin lipoprotein was significantly elevated. Serum glycoprotein values were normal. The spinal fluid ex-

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amination revealed increased total protein and gamma globulin fraction. Myxedema neuropathy and myxedema myopathy are terms suggested to define the clinical and pathologic picture seen in patients in this study.

EFFECTS ON BEHAVIOR AND CONSCIOUSNESS OBTAINED THROUGH STIMULATION OF THE RETICULAR FORMATION OF THE MACACCA MULATTA. L. D. Proctor, R. S. Knighton, J. A. Churchill and J. Bebin. First International Congress of Neurological Sciences (1957). London, Pergamon Press, 1959, v. 5 p. 180.

Further observations have been set forth, showing the effects of stimulation of the reticular formation in the macacca mulatta. Stimulation of widely spaced electrodes in the reticular formation in areas between the anterior hypothalamic nucleus and the reticular substance at the junction of the mid-brain and pons has produced in one instance hemiballismic movements and in another 'genital response.' Impairment of consciousness, as measured by impairment of the animals' efficiency in performing a psychometric test, was shown to occur in varying the placement and parameters of stimulation within these areas. Two other animals in which electrodes were placed outside of the reticular formation (caudate nucleus, pulvinar of the thalamus, in the transverse fissure above the collicular plate and in the transverse fissure immediately above the stratum zonale of the dorsal thalamus), showed only motor components as a result of stimulation using voltages up to ten times that used in stimulation of the reticular formation.

INTERRELATION OF TESTS OF INTESTINAL ABSORPTION. J. A. Rinaldo, Jr. Gastroenterology 40:86, 1961.

Tests useful in the assessment of intestinal absorption include measurement of the amount of ingested *d*-xylose in the urine, fasting serum carotene concentration, and fecal fat. The present investigation examines certain aspects of these tests, including the interrelation among them, the relation between the clinical severity of a disease of malabsorption and the variation of each test, and the determination of the most efficient application of the tests as a group. We measured fasting serum carotene concentration, 4-hour urine *d*-xylose excretion, and 72-hour fecal fat excretion in two groups of patients. The first group included functional patients having diarrhea but excreting a normal amount of fecal fat. The second group consisted of patients having steatorrhea resulting from nontropical sprue or exocrine pancreatic insufficiency or partial gastrectomy. Each patient was graded according to the clinical severity of his disease, and the grade obtained was compared to the test result. Fecal fat excretion was the only test that showed consistent progressive abnormality as the clinical severity increased, but then for only sprue and pancreatic insufficiency. A correlation was found between serum carotene concentration and fecal fat excretion, but this correlation was not sufficiently close to make serum carotene determination a useful predictor of severity of steatorrhea.

THE VARIATION IN CLINICAL RESPONSE TO MARPLAN WITH DURATION OF ILLNESS. R. R. SCHOPBACH. Am. J. Psychiat. 117:746, 1961.

This paper indicates that the effects of hydrazines upon depressions vary with the duration of the illness. A lowered serotonin metabolism in depression is also suggested. Routinely 30 mg. Marplan per day was given either in one dose or divided to spread the psychologic effect. Of the 28 patients only 6 were suffering from an acute depression. Of these 6 acute depressions 2 cleared within 1-2 days, making it doubtful that the drug was the responsible curative agent. The results in the group of 22 more chronically disturbed depressed patients were less gratifying. Thus, as with all therapies, Marplan is more effective in acute depressions but is still of definite value in those of longer duration.

*ISOLATION AND PROPERTIES OF THE DPNH DEHYDROGENASE OF THE RESPIRATORY CHAIN FROM HEART MITOCHONDRIA. R. L. RINGLER, S. MINAKAMI AND T. P. SINGER. Biochem. & Biophys. Res. Comm. 3:417, 1960.

This paper reports the first successful solubilization and partial purification of what is perhaps the most important oxidizing enzyme in all higher forms of life: the one responsible for the reoxidation of reduced DPN by way of the respiratory chain. This enzyme links the oxidation of some 95% of the oxidative events which result in energy conservation in the cell to the system of oxidative phosphorylation, which traps and transforms this energy into ATP, the pirmary chemical fuel for all synthetic processes and work performed by living cells. The paper describes various properties of the enzyme, such as substrate specificity, action of inhibitors, absorption spectrum, turnover number, etc.

*ON THE MULTIPLICITY OF LACTIC DEHYDROGENASES IN YEAST. T. P. SINGER, E. B. KEARNEY, C. GREGOLIN, E. BOERI AND M. RIPPA. Biochem. & Biophys. Res. Comm. 3:428, 1960.

This paper is a brief summary of the results of a collaborative investigation by the Department of Human Physiology of the University of Ferrara (Italy) and the Enzyme Division of this institute. It is concerned with several problems, the most fundamental one of which is the demonstration that an oxidative enzyme, specific for the D(−) configuration of lactate (D-α-hydroxy acid dehydrogenase), present only in *anaerobic* yeast, is not a precursor of the L (+) specific lactic dehydrogenase of aerobic cells (the so-called cytochrome b_2), contrary to repeated claims in the literature. This conclusion was based on a comparison of the enzymatic and chemical properties of the two isolated proteins, on the effect of growth conditions on the formation of the two enzymes, and on a study of mutant cells. The paper further reports the discovery of two new lactic dehydrogenases (which were later isolated in Detroit) and also demonstrates that, unlike generally believed, alcohol is not the only normal product of yeast fermentation but that, as in animal tissues, considerable amounts of lactate accumulate during anaerobic glycolysis.

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HEALING COMPLICATIONS WITH PLASTIC ARTERIAL IMPLANTS. R. F. SMITH AND D. E. SZILAGYI. Arch. Surg. 82:14, 1961.

The purpose of this study was to recount the incidence, causes, and treatment of healing complications (wound infections, anastomotic false aneurysms, and suture line hemorrhages) in 343 reconstructive arterial operations using plastic arterial substitutes (in 90 percent of the cases elastic woven Dacron protheses). There were 3 superficial and 11 deep wound infections. Among the cases of deep wound infection 6 serious hemorrhages occurred, and in 3 fatal septicemia developed; in most of these cases the infected prostheses had to be removed. The etiological factors in the 32 false aneurysms, 7 of which ruptured, were in order of frequency: diseased arterial wall at the site of anastomosis, defective suturing, defective prothesis, and deep wound infection; excision with reconstructive repair was unsuccessful in over half of the cases.

ARTERIOPLASTY FOR SEVERE ISCHEMIA OF THE LOWER LIMBS: INDICATIONS AND RESULTS. D. E. SZILAGYI, R. F. SMITH AND A. ESPHANI. Surgery 49:242, 1961.

The use of grafting procedures in patients with severe ischemia of the lower limbs caused by occlusive arteriopathy is clearly indicated whenever the possibility of success exists and the patient's general condition permits the intervention. The minimum technical requirement for femoropopliteal grafting in these cases is the presence of an open popliteal arterial stem with at least one functioning infrapopliteal branch. The minimum technical requirement for aortoiliac grafting is a functional common femoral arterial segment or the existence of an anatomic situation in the popliteal area described above. A successful operation will always completely rehabilitate the patient. The duration of the rehabilitation is not known but it is probably at least 2 years in two-thirds of the cases. An unsuccessful operation may precipitate amputation but, under the operative indications described, such an amputation either is inevitable or merely means the loss of a functionless limb.

THE BIOLOGIC FATE OF HUMAN HOMOLOGOUS ARTERIAL GRAFTS. D. E. SZILAGYI. Minerva Cardioangiologica Europea 8:55, 1960.

We wish to report very briefly the observations we made in course of the study of the structural alterations that take place in human homologous arterial grafts after implantation. The study was based on an experience of 350 surgical procedures utilizing such grafts and performed between January 1952 and July 1957. In spite of the excellence of their technical qualities and the brilliant early results they have generally yielded, from a long-range point of view both the human aorta and the human femoral artery suffer from serious deficiencies as homologous vascular substitutes; the femoral artery, in particular, seems intrinsically poorly suited to serve as homograft. Consequently, in our practice the use of homologous arterial grafts has been completely abandoned.

GROIN TO CALF BY-PASS GRAFT IN THE TREATMENT OF FEMORO-POPLITEAL ARTERIAL OCCLUSIVE DISEASE. D. E. SZILAGYI AND L. S. FALLIS. Minerva Cardioangiologica Europea 8:150, 1960.

The cause of the late failures after grafting procedures for the correction of femoro-popliteal arterial occlusive lesions are very briefly reviewed, and the point is made that the large majority of these failures have in the past been due to incomplete reconstruction of the arterial tree. A surgical technique is described that allows the insertion of a long femoro-popliteal bypass in an uninterrupted technical sequence. Bridging, in this manner, a longer segment of diseased artery and thus achieving a more nearly complete circumvention of the occlusive process, this operation promises to reduce the late failure rate of grafting operations for occlusive disease of the femoro-popliteal area.

MALIGNANT MEDIASTINAL TERATOMA WITH BONE METASTASES: A CASE REPORT. A. W. TEMPLETON. Radiology 76:245, 1961.

A teratoma is a true neoplasm, composed of multiple tissues foreign to the part of the body in which it arises and possessing the power of progressive growth. According to its behavior or structure, it may be benign or malignant. Clinically, malignant mediastinal teratomas are seen predominantly in males. The average age of occurrence is twenty-seven years. After onset of symptoms, the average duration of life in Schlumberger's series was five and a half months. Presenting complaints included cough, chest pain, dyspnea, hemoptysis, and engorgement of the neck veins. An unusual case of malignant mediastinal teratoma with proved metastases to the lumbar spine has been presented.

CLINICAL EVALUATION OF CANCER CHEMOTHERAPY WITH 5-FLUOROURACIL. V. K. VAITKEVICIUS, M. J. BRENNAN, V. L. BECKETT, J. E. KELLY AND R. W. TALLEY. Cancer 14:131, 1961.

5-FU was able to induce objective tumor regressions in a variety of tumors although commonly only at the expense of severe toxicity. Vigorous supportive therapy, combined with early treatment of complicating infections, reduced the hazards of therapy sufficiently so that in far advanced cases of otherwise untreatable breast and primary hepatocellular carcinomas 5-FU became a clinically useful agent. In the treatment of ovarian carcinoma, the results obtained by 5-FU therapy are comparable to those obtained with radiomimetic agents. Because of the more unpleasant symptomatic effects of 5-FU treatment, radiomimetic drugs are probably preferable. 5-FU has a narrow therapeutic range. Toxicity is regularly produced with effective doses but is manageable. The spectrum of tumors undergoing clinically valuable regressions is narrow but does include such common tumors as carcinoma of the breast, ovary, and large bowel as well as hepatocellular carcinoma.

FREEZE-PRESERVATION OF ENZYMATICALLY PREPARED TUMOR CELL SUSPENSIONS AND THEIR USE IN TUMOR TRANSPLANTATION. V. K. VAITKEVICIUS, W. L. SIMPSON AND M. J. BRENNAN. Cancer Res. 21:187, 1961.

Methods currently in use for preparation of cell suspensions for tissue culture are useful for tumor transplantation work and permit quantitative assays of factors affecting transplantation. Enzymatic digestion yields higher numbers of viable cells than the commonly used mechanical method of squeezing cells through metal meshes. Cell suspensions prepared by enzymatic digestion can be stored at low temperatures for prolonged periods of time without changing their transplantability. Tumors requiring only small inoculum sizes for transplantation can be successfully freeze-preserved by virtually any method of tissue freezing currently in use. The less "virulent" tumors studied require protection by adequate suspension media with added glycerol for successful preservation and may be injured by enzymatic digestion prior to freezing. The elimination of stroma in such cell suspensions allows more adequate quantitation of the transplanted tumor cells and decreases the variability in the latent periods and in the percentages of successful transplantations. The best over-all preservation of mouse tumor cells for transplantation appeared to follow enzymatic isolation, suspension in the Eagle-Earle solution and glycerol, followed by addition of serum before freezing. The possible applicability of these technics to work with human tumors is now being tested.